Major Compulsory Revisions

TITLE
- consider to adapt the Title to the Results. In this study the authors do not demonstrate the adenoma-carcinoma sequential process in the salivary gland.

ABSTRACT
- consider to remake Methods: they are not explained

Results:
- consider to start with “We established and characterized five cell systems (designed as SM-AP1 to SM-AP5) from a benign pleomorphic adenoma of the parotid gland.”
- consider to delete “immunohistochemically, polygonal shaped” in line 2, “spindle shaped” in line 4, “that the cells had not only” in line 6, “with average numbers of 108 chromosomes but also” in line 8 and replace “and” between “ploidies” and “various”.

Conclusions:
- do the authors conclude that........

“This study is an in vitro demonstration that certain cell types from pleomorphic adenoma are able to clone and survive for long term and develop subcutaneous tumors in nude mice.
The histological features of squamous cell carcinoma from the transplanted cell systems in nude mice might suggest a secondary onset of malignancy from a pre-existing benign adenoma.”

BACKGROUND
- consider to delete till line 7. Start with “Among....”
- “to start with “Pleomorphic..... in line 21. Include “and molecular “after “Cytogenetic” and delete the words “in spite of their benign natures” in line 22.
- consider to delete “such” and “as” in line 25, replace “These” instead of “The” and delete the words” found in these studies” in line 26.
- consider to remake the text from line 33 to the end. The authors should
consider previous data of established cell lines from human pleomorphic adenoma (bibliography 23-25, 14, 26, 8-10, 27-31) in outlining the aim of their work.

RESULTS
- consider to include the subtitle “Tissue sample histology” and the text till line 6 (Fig.1B). Then follow with the subtitle “Establishment....”
- consider to delete in line 1-2 (used.....culture).
In : Establishment....:
- consider to delete (line 7) “and ... proliferation” and (line 8) “was accelerated” and the word “regularly”.
In: Chromosome analysis:
- consider to insert after the first paragraph (line 3), the paragraph in line 19-20, and the words “(Figure 3A-f )”.
- consider to delete (line 3-4) the words “that they had”.
- In line 6 add the words “Figure 3B represents SM-AP5 stemline karyotype”.
- consider to delete (line 6-7) the words “and cells in the primary culture”.
- at the beginning of line 13 put “the origin....” and “...was”...
- in line 20, delete “They” and replace with “ Primary cultured cells”.
- in line 22 delete “Figure 3A-f and “Furthermore”.
In Screening.....:
- in line 8 , put a full stop after “Figure 4A-a” and star with “BAC clones....”
In Xenografts....:
- consider to adapt accurately the histological description to the figures. Include in results part of the text of Figure 6 (from “ SM-AP formed...”). Arrows in the figures are necessary.

DISCUSSION
- consider to delete “which....benign” in line 2-3 and replace “benign” before “parotid” in line 2. The second paragraph should be discuss at the end. Put “previously” after “We have” in line 8.
The paragraph in line 13 to 17 should be discuss at the end.
- consider to summarized the text from line 18 to line 35 in the background. The same text could be use in the Discussion keeping in mind the results obtained in this study(transplanted tumors with characteristics of squamous cell carcinoma)
- consider to make clear the part concerning the histological phenotype of transplanted tumors (line 40-64)
- from line 65: consider to star with “The”, put “in addition to” after “showed” and
delete “also” in line 65; delete “shared by each other”and replace “of which”,
delete “among the chromosomal abnormalities” in line 67-68; delete “them” and 
replace “all of the clones” in line 69. Delete “were successful in restricting “ and 
replace “were able to restrict” in line 71. Delete “in these cell systems in line 72. 
Consider to delete the paragraph in line 78-80 and replace it with “A role of p16 
gene in the secondary onset of malignancy in pleomorphic adenomas has been 
hypothesized by Suzuki & Fujioka.” (34). Delete from “these …to 9p13” an 
replace with “ 9p13 rearrangements “ in line 81 and put (34) at the end of the 
paragraph (line 84).

- consider to delete the text from line 87 to 94. Start with “None” in line 95 and 
replace “the” instead of “these” in line 96. After “8q12” follow with “where PLAG1 
, one of the most extensively investigated genes in pleomorphic adenomas is 
located.

- consider to delete the text from line 101 to line 110 and replace with 
“Abnormalities such as…………….found in our cell systems constitute a new 
finding in pleomorphic adenoma.

- consider to remake the text from line 111 to the end . It is too long. Try to 
discuss your results primarily.

Minor Essential Revisions

METHODS

- consider to delete “s” in the subtitle “samples”
- “ “ (line 2) who………months.
- “ “ (line 9-10) Microscopic ………….adenoma.
- consider to put together “Primary culture and cloning”. Consider to delete in line 
11-12 “and…..times” and put “passaged” instead of “their …performed” in line 14.

Immunohistochemistry, consider to substitute in line 2, “They” with “The cells”and 
delete in line 6 “used….study”.

FIGURE LEGENDS

Figure 1
- consider to delete the word “Histopathologically” in line 6.

Figure 2
- put “Immunocytochemistry” instead of Immunohistochemistry in line 1.

Figure 3
- consider to delete lines from 9 (SM…) to 13.

Level of interest: An article of limited interest
Quality of written English: Needs some language corrections before being published

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'