Reviewer’s report

Title: Dual tracer evaluation of dynamic changes in intratumoral hypoxic and proliferative states after radiotherapy of human head and neck cancer xenografts using radiolabeled FMISO and FLT

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Reviewer: Hideo Tsukada

Reviewer’s report:

Major Compulsory Revisions

1) In general, the aim of this study seems to be unclear why the authors used FaDu cells which were characterized to have moderate radiosensitivity as described in line 286 to see the dynamic changes in levels of hypoxia and proliferation in cancer after single radiation therapy. Before the authors conducted this study, references # 26, 27, 28, 29, and 30 had already indicated the requirement of fractionated radiation for more than 7 days to induced the reoxygenation after radiation.

2) In Abstract and Materials and Methods parts, the aim to use pimonidazole should be explained. In addition, why did the authors conducted only immunohistochemical assessment for hypoxia, not for proliferation such as Ki-67 staining?

3) In Abstract, Introduction, Discussion, and Conclusion parts, the authors emphasized that “Evaluation of tumor hypoxia and proliferation is useful for optimizing an individualized treatment strategy.” in lines 51, 115, 337, and 348. However, as pointed out in comment #1, FaDu cells used in this study did not support the usefulness of the evaluation of tumor hypoxia and proliferation for individualized treatment strategy. The authors should exclude this sentence or apply other tumor cells with more radiosensitivity to reveal changes in hypoxic level within planed period after single-dose radiation.

4) For Figure 3, the authors implied that hypoxic regions determined by 18F-FMISO were distributed in the central part of the tumor tissue in line 257. However, it cannot be acceptable because Figure 3 as well as Figure 5A showed the hypoxic regions also in the peripheral regions in almost all conditions.

5) In Figures 2 and 4B, there were no significant dose-responses both in tumor growth and 3H-FLT uptake. If they used higher radiation doses, are there any possibilities to detect the reoxygenation even after the single radiation? Did the authors apply appropriate radiation doses to confirm their statement regarding the usefulness of the evaluation of tumor hypoxia and proliferation?

6) In Discussion part, the authors discussed too much issues based on their results. The results that the authors obtained were only the reduced 3H-FLT uptake and no significant changes in 18F-FMISO uptake as well as pimonidazole immunohistochemical activity. All statements regarding the usefulness of the
evaluation of tumor hypoxia and proliferation for individualized treatment strategy should be excluded, because their results did not support this notion at all.

Minor Essential Revisions

None.

Discretionary Revisions

Even without data about Ki-67 staining in this manuscript, Authors’ contribution stated “CNF drafted the manuscript and performed the experiment, all data analysis, immunohistochemical staining of pimonidazole and Ki-67.” in line 366.

Level of interest: An article of insufficient interest to warrant publication in a scientific/medical journal

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.