Reviewer's report

Title: Induction of plasminogen activator inhibitor type-1 (PAI-1) by hypoxia and irradiation in human head and neck carcinoma cell lines

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Reviewer: mike robbins

Reviewer's report:

General
This manuscript describes studies in which the authors observe that short- and long-term hypoxia, as well as radiation, can upregulate PAI-1 levels in BHY and FaDu squamous cell carcinoma of the head and neck (SCCHN) cell lines. The authors go on to suggest that the radiation-induced upregulation of PAI-1 in head and neck tumors could contribute to the poor outcome of patients presenting with this cancer. A major weakness of the manuscript is that none of the data presented support this conclusion, and thus the Discussion is speculation rather than objective data.

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

Methods
There are a number of technical concerns that should be addressed. The reoxygenation experiments are interesting, but it is unclear as to why the authors chose to change the medium after 24h of hypoxia. This does not appear to have any physiological relevance, and needs to be justified if this is to be continued.

In the Western Blot analyses, the authors state that they use only 5 µg of cell lysate to detect HIF-1α. This seems surprising, since in the literature it seems more common to use in the order of 30 µg of nuclear protein.

Results
Influence of hypoxia on cell viability: The claim that prolonged hypoxic exposure leads to increased detachment without a significant increase in viability of the adherent cells does not make sense. It would be simpler to state that hypoxia caused 30% death in the BHY cells and 14% death in the FaDu cells.

Figure 2: There are a number of concerns with this figure. The authors conclude that phalloidin staining was unchanged with hypoxia, and yet it appears as if the red staining decreases under hypoxia, particularly in the FaDu cells. Although the figure shows qualitatively a time-dependent increase in PAI-1 staining under hypoxia, it is not quantitative and should be accompanied by Western Blots to show increases in PAI-1 immunoreactive protein. Further, the API-1 staining appears to shift from cytoplasmic to nuclear, and yet there is no explanation of this in the text.

The use of ELISA to determine PAI-1 stability appears inappropriate. This should be repeated using 35S methionine-labeling.

In Figure 5, the authors show data suggesting that PAI-1 levels in the media decrease after reoxygenation, and yet in Table 1 the data indicate no change in PAI-1 levels 1 day after 24 h hypoxia. These findings appear contradictory and require explanation.

Discussion
The data are limited to showing that, as in other cell lines, both hypoxia and radiation can upregulate PAI-1 gene expression and protein. Moreover, there are no studies aimed at testing the hypothesis that this radiation-induced increase in PAI-1 protein could play a role in the poor outcome for these patients.

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

Figure 1: It is surprising that the authors did not note a time-dependent increase in HIF-1α protein during hypoxia. Further, it is unclear as to the concentration of DFO used here; the increase in HIF-1α with DFO appears modest.
Combine Fig 2, Fig 3 and Fig 4.

Discretionary Revisions (which the author can choose to ignore)

**What next?:** Reject because too small an advance to publish

**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 I have no competing interests