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Hypoxia-inducible Factors Are Already “Active” in the Von Hippel-Lindau–deficient Renal Cell Carcinoma-4 Cells

To the Editor:

I read with great interest the article by Benzonana *et al.*¹ investigating the effect of isoflurane on the cellular function of renal cell carcinoma (RCC)-4 cells, which are derived from human RCC.

I think that there are several issues in this study that deserve comments.

In this study, the authors exclusively used the RCC cell line, namely RCC4 cells. It is well known that the tumor suppressor gene Von Hippel-Lindau is deficient in these cells. Because Von Hippel-Lindau plays a critical role in the regulation of stability of hypoxia-inducible factor (HIF)- α proteins in cells, HIF-1 α and HIF-2 α proteins are “stabilized” or overexpressed in RCC4 cells even under 20% O₂ conditions^{2,3} and the expression is not increased under 1% O₂ conditions.^{2,3}

However, as demonstrated in figure 1 of their study, HIF-1 α and HIF-2 α proteins only barely expressed in RCC4 cells under 20% O₂ conditions, as shown by them. I would like to ask the authors to explain the discrepancy between this and the original report.²

One of the features of malignant tumors is the hypoxic region in them, where the activation of HIFs is observed.⁴ Thus, I think that it is essential to investigate the effect of isoflurane under such hypoxic conditions. In this study, the authors exclusively performed the assays under 20% O₂ conditions or normoxic conditions. However, we reported that the volatile anesthetic halothane inhibited hypoxia-induced HIF-1 activation in Hep3B cells⁵ and that isoflurane suppresses HIF-2 activation under hypoxemic hypoxia in mice.⁶ The ontology behind this discrepancy should be clarified.

The authors argue that isoflurane could promote the expression of HIF-1 α and HIF-2 α , which are subunits of the transcription factors HIF-1 and HIF-2, respectively, and that isoflurane might enhance the cellular activities that are associated with a malignant phenotype in the cells in HIFs-dependent manner. I totally agree that both HIF-1 and HIF-2 play a critical role in cancer progression by providing pro-cancerous microenvironment. In figure 4 of their study, the authors indicated that 2% isoflurane treatment enhanced cell growth or proliferation by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. However, there is no consensus that HIFs act as

an “oncogene.” I wonder by which molecular mechanisms the authors think HIFs enhance the cell proliferation.

In addition, I think that the argument that isoflurane enhances tumorigenesis in a HIFs-dependent manner is under discussion. No experimental evidence was demonstrated to indicate that the “induction” of HIFs- α protein is essential for the increases in RCC4 proliferation as examined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay in their study.

Usually, the patients are exposed to the anesthetics only during the surgical treatment. That means the duration is not so long and transient. Do the authors have any experimental results on the reversibility of isoflurane on HIFs- α expression?

Finally, the authors described that LY294002 is a protein kinase B (Akt) inhibitor. However, almost all molecular biologists think that LY294002 is a phosphoinositide 3 kinase inhibitor.⁷

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Competing Interests

The author declares no competing interests.

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