Reviewer's report

Title: Enolase/MBP-1 Mediate the cellular Response to Hypoxia

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Reviewer: Carine Michiels

Reviewer's report:

The work by Sedoris et al aimed to understand the role of the two proteins encoded by the enolase mRNA in the response of changes in glucose concentration under hypoxia conditions. While numerous measurements have been performed, there were no intervention experiments that could have led to the understanding of the ROLE of these proteins. Only correlations were observed and even for that, there were often poor (see below). Indeed, there is no correlation between a decrease in MBP-1 protein level and an increase in c-myc expression either at the mRNA or at the protein level. Furthermore, there is no correlation between c-myc mRNA level and protein level (especially at the high glucose concentration). Similarly, there is no correlation between the mRNA and the protein levels of enolase (again especially at the high glucose concentration).

Moreover, sometimes in the text, the results obtained under normoxic conditions are mentioned but there is no actual data here (except for growth and ROS production). The authors should show the results obtained for the three glucose concentrations under normoxic conditions, in order to appreciate the differential effect of hypoxia in comparison to the one of glucose concentration.

Finally, this is very strange that HIF-1alpha protein stabilization was not detected at shorter time points. Indeed, at 1 % O2, HIF-1alpha is stabilized within one hour. A complete kinetics should be performed. Using cytosolic extracts would be easier (HIF-1alpha is a very unstable protein and may have been degraded during the nuclear extraction procedure). If the authors want to conclude that ROS are involved in regulating HIF-1alpha protein stabilization, again, an intervention experiment should be performed, for example using an antioxidant.

Major comments

- The title should be changed: there is no proof at all that enolase/MBP-1 mediate the cellular response to hypoxia in this work. Moreover, this work compared the effect of different glucose concentration while there is no comparison between normoxia and hypoxia per se.

- The well-known role of HIF-1 in relating the expression of glycolytic enzymes including enolase is not mentioned in the “background” while it is very important to understand the rationale of this work.

- There should be some explanation, either in the “background” or in the “discussion” part, regarding the mechanism underlying the alternative translation of the enolase mRNA that leads to two different proteins.
- The description of figure 4C should come just after the description of figure 4B.

Minor comments
- page 3, line 5 : “HIF-1 affects counteract c-myc…” should be HIF-1 counteracts c-myc…”
- page 4, line 16 : “Its overexpression in tumors at has been…” should be “Its overexpression in tumors has been…”
- page 8, last line : “attenuatedMBP-1 expression f” needs to be corrected.
- Table 1 is not referenced in the text. There is no legend for this table/figure.
- figure 1 : “Effect of hypoxia and altered glucose concentration” : the glucose concentration is not “altered”, it is different.
- all figures : * and # are used to define statistical difference but it is not mentioned to what the values are compared. If it is to time 0 (which should be the case !), there are instances where # seems very doubtful (for example in figure 7, Hyp 1 nm 48h, Hyp 5 mM 12h, Hyp 25 nM 6h, 48 h).

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests:

I declare that I have no competing interests'