



## SDI FINAL EVALUATION FORM 1.1

### PART 1:

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| Journal Name:            | Journal of Advances in Medical and Pharmaceutical Sciences                |
| Manuscript Number:       | Original Ms_JAMPS_29407                                                   |
| Title of the Manuscript: | Aspects on hypoxia-tolerance: rescue mechanisms for surviving oxygen lack |
| Type of Article:         | Original Research Article                                                 |

### PART 2:

| FINAL EVALUATOR'S comments on revised paper (if any)                                                                                                                                                                                                                   | Authors' response to final evaluator's comments                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |                                                                                                                                                                                                                                                                                                                   |
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| <p>The manuscript is still not readable. There are still a significant number of inconsistencies. For example the use of H1 and H2 should thereafter be consistency. Additionally, list of correction which has been suggested versus does not include everything.</p> | <p>In your first report all your comment have been fulfilled and the English of the manuscript has been essentially changed. The words of thanks are not sufficient for your fruitful and constructive points.</p> <p>Please, would you kindly the responses to your comments below this report</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |                                                                                                                                                                                                                                                                                                                   |
|                                                                                                                                                                                                                                                                        | Reviewer's comment                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           | Author's comment (if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)                                                                                                                                     |
|                                                                                                                                                                                                                                                                        | <p><b>Summary</b><br/>The researchers' main aim was to separately evaluate if both hypoxia inducible factor-1 (HIF-1) and HIF-2 play a role in hypoxic response in tumors, whether they influence each other and if both influence glucose metabolism (glycolysis) during a hypoxic state. Cells devoid of HIF-1 (786-O from patients with renal carcinoma with mutant VHL gene) were used to study HIF-2 inducible genes. RCC-4 cells were used for analysis of both HIF-1 and HIF-2 inducible genes. The main challenge they noted was that both subtypes of HIF are co-expressed in most cell lines.</p> <p>Various levels of oxygen tension below 21% and/or alternatively hypoxia-mimetic agents were used. The study was able to confirm what is already known that HIF-1alpha and not HIF-2alpha influences hypoxia inducible genes. Deficiency of HIF-1 makes tumor cells unresponsive to hypoxia and no induction of glycolytic genes. Additionally, there is no interaction between HIF-1alpha and HIF-2alpha. Furthermore, the study was able to confirm that indeed, HIF-1 and not HIF-2alpha influences glycolysis and thus glucose metabolism and related response inducible genes in tumors.</p> <p>Although the area of research is of interest and cotemporary, one's concern is that the abstract, introduction and method sections of the manuscript is not well written and the authors did not pay attention to details. The manuscript is difficult readable and is therefore not acceptable in its current state.</p> | <p>Thank you Mr. Professor reviewer for your fruitful comments:</p> <p>The title has been changed to be:</p> <p><b>Effect of hypoxia inducible factor on hypoxia inducible gene expression and glycolysis.</b></p> <p>Also, substantial changes have been done all over the English of the entire submission.</p> |



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|  | <p><b>Title</b><br/>Assessment: Ok.<br/>Although one is not sure if tumor growth was studied. Perhaps <b>'Effect of hypoxia inducible factor on hypoxia inducible gene expression and glycolysis'</b></p> <p><b>Abstract</b><br/>Assessment: Not well written<br/>This section can be significantly improved. It needs major revision of content and grammar. The main aim of the study should be spelled out and the abstract must be focused on issues addressed in the current study. HIF-1alpha Hypoxia does not cause cancer but promotes development of strategies to survive a hostile environment in cancers such as during metastatic pathway by inducing neovascularization, switch to glycolysis as the main source of energy, switch off of mitochondrial processes and enhance ability of cancer cells to survive an acidotic environment. That HIF-1alpha is constitutionally expressed in most cells and that it rather than HIF-2alpha is able to cause an increase in transcription of genes which regulates glycolytic process and thus influence glucose utilization by tumors during a hypoxic state.<br/>All statements in the abstract need to be revised either because they are incorrect, vague or grammatically not appropriately phrased.</p> <p><b>Introduction</b><br/>Assessment: Not well written for the following reasons:<br/>a) The following statements are factually incorrect<br/>i. That HIF-2 alpha is the 2nd organ of the hypoxia-inducible factor family.<br/>ii. Nomenclature for the three known HIFs should be used appropriately.<br/>iii. The nomenclature of HIF-2 alpha is compatible with HIF-1 alpha.<br/>iv. That HIF-2 alpha is highly induced with hypoxia in organs such as lung, liver, pancreas, brain, heart and intestines. But is a yes for HIF-1alpha.<br/>v. Hetero-dimerization followed by entry into the nucleus and then transcriptional activation of hypoxia inducible genes (DNA) is reported for HIF-1alpha and HIF-1beta, and not cross binding with HIF-2alpha.<br/>vi. HIF-2alpha has been studied in normoxic conditions.<br/>b) There is not a single statement which would not need revision to improve English grammar.<br/>c) Discussion on structural similarities and differences between HIF-1alpha and HIF-2alpha was not the key focus of the study and should therefore not be over emphasized, including a repeat of statement regarding similarity of 48%.<br/>d) Consistency is necessary. Not to alternate the use of cancer and tumor.<br/>e) The last three sentences in the section (bottom of Page 2) are not merging well with previous statements</p> | <p>The abstract has been substantially changed.</p> <p>All these incorrect sentences have been corrected</p> <p>The manuscript has been substantially corrected.</p> <p>The similarity has been changed to become</p> |
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|  | <p>and the theme. It may suggest “cut and paste”.</p> <p>f) To try to find alternate phrases for most statements.</p> <p><b>2. Materials and Methods</b><br/>Assessment: Ok.</p> <p>2.1. Needs revision of English grammar.</p> <p>a) The authors may need to further fragment some of the statements.</p> <p>b) We chose instead of choose.</p> <p>c) “Infected in our study” is not complete.</p> <p>d) In instead of “under” 1.5% oxygen.</p> <p>e) Human microvascular endothelial cells from the lungs were sourced from and grown in EGM-2MV. 3</p> <p>2.2. Needs revision for clarity.</p> <p>a) Nucleotides.</p> <p>2.3. Suggested corrections</p> <p>a) We cultivated 786-O WT-8 cells in 21% oxygen for 24 hours. Were heterodimers of HIF-1 and HIF-2 (i.e. alpha and beta units studied)? <b>Correct nomenclature to be used.</b></p> <p>b) DNA-microarray analysis.</p> <p>c) A single cDNA was generated.</p> <p>d) Similar versus comparable amount.</p> <p>e) Dissolved versus resolved.</p> <p>f) Immersion versus blockage.</p> <p>g) Two tests were conducted in.</p> <p>h) Initially....test array.</p> <p>i) Nuclear extracts were.</p> <p>j) 3% fat free milk.</p> <p>k) Peroxidase.</p> <p>l) Horse radish.</p> <p>m) Reverse transcriptase.</p> <p>n) pcDNA3 expressing vector.</p> <p>o) Underlined letters indicate start codon for both...and.....</p> <p>p) Tag was inserted at the</p> <p>q) 10-amino acid tag.</p> <p>r) Alinine.</p> <p>s) Consistency regarding HIF-subtypes nomenclature.</p> <p>t) To check spacing of words such as 200 ng (200ng).</p> <p>u) A total of 400 1 of the medium (meaning?).</p> <p>v) Absolute cell count versus call and differentiation.</p> <p><b>Results</b><br/>Assessment: Ok<br/>Some revision is required.</p> <p>a) Please lower case and consistency with numbering.</p> <p>b) Improve grammar.</p> <p>c) A 6-whell plate.</p> <p>d) A murine sarcoma virus galactosidase.</p> <p>e) Surrogate of versus function of.</p> <p>f) For analysis of glucose concentration.</p> <p>g) The amount of glucose was measured using.</p> | <p>14% attached as it is confirmed by an ithenticate report.</p> <p>These three sentences has been deleted.</p> <p>All these incorrect sentences have been corrected.</p> <p>All these incorrect sentences have been corrected</p> |
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|  | <p>i. Perhaps contribution of HIF-1alpha as compared to HIF-2alpha is well known.</p> <p>ii. Leave out "a result that is consistent with previous reports" in Page 8 (top).</p> <p>iii. Similarly, leave out "Importantly" and start with 'No'.</p> <p>iv. And, "Consistent with their VHL status".</p> <p>v. Expression of genes such as ... was reduced by hypoxia (remove data not shown).</p> <p>vi. Some of details included may be most appropriate if moved to the discussion section of the manuscript.</p> <p><b>Discussion</b><br/>Assessment: Yes.<br/>Need some revision<br/>a) Grammar and spelling check.<br/>b) Consistency with nomenclature of HIF subtypes.</p> <p><b>Conclusion</b><br/>Assessment: Yes<br/>a) Replace present work "shows" with "confirms" as it was already known (<i>Robey et al, 2005. Neoplasia; Raval et al, 2005. Molecular and Cellular Biology; Papandreou et al, 2006. Cell Metabolism.</i>).</p> <p>b) Rephrase recommendation: Further studies to determine the role of HIF-3.....</p> <p><b>Overall assessment</b><br/>The study is good and relevant. However major revision of the Abstract, Introduction and Results sections is necessary to make it acceptable. It is not acceptable for publication in the current state.</p> <p><b>Ethical issues : Could not detect obvious ethical issues.</b></p> |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
|  | English grammar especially in the abstract, introduction and result section.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
|  | Need to clarify the meaning of HIF-1 and HIF-2 in the context of subunits.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | Thank you. This has been done. The following has been added: "Moreover, HIF1 is, in general, a protein "dimeric-complex one" which makes an essential part when oxygen concentrations tend to be sufficiently low, Thus hypoxia. HIF-1 is among the characteristic genes implicated in the homeostatic physiological mechanisms, which can raise vascularization in hypoxic domains such as localized ischemia and tumors. HIF-1 is a reproduction-factor for some decades of target genes; it is also major for immunological responses and is a crucial physiological regulator of homeostasis, vascularization, and anaerobic metabolism." |