Reviewer's report

Title: ALK1 signalling analysis identifies angiogenesis related genes and reveals disparity between TGF-b and constitutively active receptor induced gene expression

Version: 2 Date: 7 March 2006

Reviewer: Carmelo Bernabeu

Reviewer's report:

General

The authors have made an important effort and have clearly improved the manuscript following the referees’ suggestions. However, some issues remain to be clarified. It is essential to address some minor and major modifications before publication in BMC Cardiovascular Disorders.

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

1. Throughout the manuscript and specially in Methods. The spacing between numbers and concentration/amount (for example 10mM versus 10 mM or 5ml versus 5 ml) must be unified.
2. Throughout the manuscript, “et al” should be in italics
3. Page 2, Abstract, Results item, lines 4 and 5. It would be more informative if it is stated that the evaluation was performed in “human” endothelial cell types.
4. Page 5, Methods, Cell Culture and Growth factor item. “endothelial cell growth factor” Are the authors referring to PD-ECGF/TP/ECGF-1, EGF, VEGF…? Please, specify.
5. Page 5, Methods, Recombinant Adenovirus item, line 2. “….possesses; one loxP …." should be “….possesses one loxP ....”
6. Page 6, Methods, line 5. “Stock concentrations (viral particles [vp] per ml were…” should be replaced by “Stock concentrations (viral particles [vp] per ml) were…"
7. Page 6, Methods, Viral infection and Transfections item. “Transfections” should be deleted from the heading as the LCLC transfections are no longer included in the manuscript.
8. Page 6, Methods, Viral infection and Transfections item, last paragraph of the item. This paragraph is referred to LCLC cells and must be deleted as all the LCLC related material is no longer included in the manuscript.
9. Page 6, Methods, Viral infection and Transfections item, line 1. “In order to test whether HMEC-1 cells can be infected by adenovirus, cells were infected with....” should be replaced by “In order to test whether HMEC-1 cells can be infected by adenovirus, cells were incubated with....”
10. Page 7, Methods, second paragraph, last two lines. “......seen at; http://data.cgt.duke.edu/Andreas.php.” should be replaced by “......seen at http://data.cgt.duke.edu/Andreas.php.”
11. Page 8, Methods, lines 4-7. “Where possible RNA specific assays were selected which are depicted by _m1 suffix. For CHOP _g1 suffix signifies that the assay may detect DNA and for ID1 the _s1 suffix indicates both the primers and probe are in a single exon.” This explanation fits better in the legend to Table 2.
12. Page 8, Methods, line 8. Please define FAM. Green fluorescent label FAM=Carboxyfluorescein?
13. Results, page 9, line 8. “displayd” should be “displayed”
14. Results, page 9, last paragraph, line 4. “c-my” should be “c-myc"
15. Results, page 10, third paragraph, line 6. “(see Table 4))” should be “(see Table 4).”
16. Results, page 10, last paragraph, line 3. “(see Table.” should be “(see Table 5).”
17. Results, page 11, first paragraph, line 3. “transcriptionfactors” should be “transcription factors”.
18. Results, page 11, third paragraph, line 4. “(see Table 5).” should be “(see Table 6).”
19. Results, page 11, last paragraph, lines 3 and 4. “…..there is any differences…..” should be either “…..there are any differences…..” or “…..there is any difference…..”

20. Results, page 12, line 5. “…..24 hours was almost…..” should be replaced by “…..24 hours were almost…..”

21. Results, page 12, last paragraph, line 2. “CD148” does not appear as such in the cited Figure 1, but as HPTPeta. For consistency, only one gene name should be used.

22. Discussion, page 15, last paragraph, line 7. “endoglin” should be in italics.

23. Discussion, page 16, line 5. “…..24 hours was almost….” should be replaced by “…..24 hours were almost…..”

24. Table legends, page 25, Table 1. “oligonukleotides” should be “oligonucleotides”

25. Table legends, page 25, Table 1. “Primer sets were designed across exon-intron boundaries…” Perhaps is more correct to say that “Primer sets were designed across exon-exon boundaries…” as no intron sequence is present in cDNA.

26. Table 2 heading, page 25. “List of genes analysed by QR-PCR and the ABI Assay on Demand used.” As such it is unclear and should be modified. Assays-on-Demand™ is a trade mark from Applied Biosystems, but not Standard English.

27. Table legends, page 25, Table 2. See item #11

28. Table legends, page 25, Table 2. The title is confusing. Please, modify to clarify avoiding reiterative words.

29. Table legends, page 26, lines 2 and 10. “assesed” should be “assessed”

30. Table legends, page 26, Table 8. Font size should be unified.

TABLES AND FIGURE

31. Table 1, heading of the right column. For temperature, “Temp” is a better abbreviation than “Tmp”

32. Table 3. Please revise spacing between words.

33. Tables 4 and 5. The heading of the left column should be unified in both tables for consistency “Gene and gene number”?

34. Table 5, left column. “Branced-chain aminotransferase 1” should be “Branched-chain aminotransferase 1”

35. Figure 1, both panels. “DNJB1” should be “DNAJB1”

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

36. Page 2, Abstract, Conclusions item, line 4. “In addition, the results suggest cell type specific ALK1 and….” This sentence must state the specific cell lineage. For example, “In addition, the results suggest endothelial cell type specific ALK1 and….”

37. Page 4, line 3. “Both endoglin and ALK1 are predominantly expressed by endothelial and smooth muscle cells” As opposed to endoglin, this referee is not aware of reports demonstrating the expression of ALK1 on smooth muscle cells. In addition, the present study only deals with endothelial cells. Therefore it would be more appropriate to delete “smooth muscle cells” and modify the sentence as follows, “Both endoglin and ALK1 are predominantly expressed by endothelial cells”.

38. Page 4, third paragraph, lines 2-4. “To understand this requires elucidation of their function in cell adhesion, migration, and importantly gene regulation.” This sentence should be rephrased.

39. Page 4, last paragraph, lines 7-9. “For the vascular disorder HHT, at least 14 genes are reported to be involved in angiogenesis, vascular disorders or the homeostasis of the vascular system.” The meaning of this sentence is unclear. Perhaps the authors mean to say “Consistent with the vascular disorder HHT, at least 14 genes are reported to be involved in angiogenesis, other vascular disorders or the homeostasis of the vascular system.”

40. Page 5, Methods, Cell Culture and Growth factor item. A short description of the cell lines HMEC-1 (derived from human microvascular endothelial cells ?) and ECRF24 (derived from human umbilical vein endothelial cells as indicated in legend to table 6?) is required. This is a relevant issue in the manuscript as the authors state that their results vary among the different endothelial cell
types.
41. Page 7, Methods, second paragraph, last two lines. “……seen at; http://data.cgt.duke.edu/Andreas.php.” The GeneChip data generated can not be seen at the webpage indicated (on March 7th, 2006). This must be corrected.
42. Page 8, Methods, first paragraph-lines 2 and 3 and second paragraph-line 2. Assays-on-Demand™ is a trade mark from Applied Biosystems, but not Standard English. The corresponding sentences should be modified accordingly. The authors should take into account that the manuscript is intended for a cardiovascular research readership, and they may not be familiar with the specialized gene-expression jargon.
43. Results, page 10, third paragraph, last two lines. “….but their subsequent up-regulation after 24 hours might be an indirect effect by ALK1 signalling or an effect of prolonged ALK1 signalling” This explanation is confusing as a prolonged ALK1 signalling might also induce ALK1-dependent indirect effects. This sentence should be modified/clarified.
44. Results, page 11, first paragraph, lines 2-5. “The identified early response genes can be roughly divided into four functional groups……” Are KPNA3, BCAT1, and NF2 excluded from this classification? If so, please clarify.
45. Results, pages 11 and 12, “TGF-b1 regulated gene expression of ALK1 response genes in HMEC-1 cells” item. The authors wonder whether there is any difference between high or low concentrations of TGF-b1, which reportedly activate either the ALK5 or the ALK1 pathway. Then, the authors incubate the cells with 0.5ng/ml or 4ng/ml TGF-b1 for 16 hours and 24 hours, respectively. However the authors do not justify the selection of this time points, especially when the differences between ALK1 or ALK5 pathways have been reported by other authors when low TGF-beta concentration is applied for 2-4 hours, while in these experiments the authors incubate for as long as 16 hours. Thus, it is not surprising to find that the expression profiles of TGF-b1 treated cells after 16 hours or 24 hours are almost identical regardless of TGF-b1 concentration. Therefore, data from the time point at 16 hrs could be deleted from the table without affecting the conclusions. In addition the authors should explain the lack of differences in the data by commenting the possibility that the selected incubation time conditions are probably not optimal to see the expected differences.
46. Results, page 11, third paragraph, last three lines. “These results suggest that there is a notable endothelial cell type as well as endothelial cell lineage difference in ALK1-induced gene expression.” As written this sentence is confusing since all the endothelial cells belong to only one cell lineage. Probably the authors wish to modify the sentence as follows “These results suggest that within the endothelial lineage there is a notable cell type difference in ALK1-induced gene expression.”
47. Results, page 12, lines 9 and 10. “…..being up-regulated after 16 hours with decreasing expression after 24 hours.” This sentence is misleading because c-myc expression does not decrease after 24 hours, but upregulates less than at 16 hours. The authors can probably replace the above sentence just by “…..being maximally up-regulated after 16 hours.”
48. Discussion, page 15, second paragraph, lines 3 and 4. “Cell type/cell lineage differences were also observed for…..” According to previous comments (see above), this sentence could be replaced by “Cell type differences were also observed within the endothelial lineage for…..”

TABLES AND FIGURE
49. General comment on Tables. Addition of an overall and simple heading title for each Table would be very informative and helpful to the reader.
50. Table 7. As indicated in previous item #45, the information provided at 16 or 24 hr of TGF-beta treatment is basically the same. Accordingly, one of these two time points (16 hr) could be deleted without affecting the conclusions of the manuscript.
51. Figure 1. The purpose of this figure is to compare the expression response of each gene at high versus low TGF-beta concentration. However, this comparative analysis is difficult to perform given the separation of the results in two different panels. One has to go back and forth from panel A to panel B finding the name of the specific gene. However, this comparison should be much easier if data from each gene at high or low TGF-beta are placed together. Of course, then no panel classification would be needed.
Discretionary Revisions (which the author can choose to ignore)

**What next?** Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** No

**Declaration of competing interests:**

I declare that I have no competing interests