Reviewer's report

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

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Reviewer: Carmelo Bernabeu

Reviewer's report:

General
This manuscript intends to identify genes regulated by the TGF-beta receptor ALK1 that is involved in angiogenesis, as exemplified by the fact that mutations in ALK1 gene cause the vascular disorder known as Hereditary Hemorrhagic Telangiectasia (HHT). The authors use a recombinant constitutively active ALK1 adenovirus to infect endothelial (cell lines HMEC-1 and ECRF24; HUVECs) and non-endothelial (LCLC) cells. Of interest is the finding that some genes upregulated by ALK1, such as IL-8, ET-1, ID1, or CD148, are reported to be involved in angiogenesis. Although the subject of this manuscript is of potential interest, the numerous results obtained are not always consistent and no clear conclusions are drawn from these studies. Also, the manuscript is too lengthy and some of the results presented only add confusion to the manuscript. The presentation of Results needs clarification and reorganization. Shortening of the whole manuscript is required, focusing only on the coherent data of endothelial cells.

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)
1. The authors present contradictory gene expression patterns between different endothelial cell types. Further complication is provided by the use of the non-endothelial cell line LCLC. The study should be restricted to the analysis of endothelial cells. Data from Figure 2A-F do not really provide any clue about the divergent gene expression data and must be deleted.
2. The authors present contradictory findings between constitutively active ALK1-, constitutively active ALK5-, or TGFbeta-induced gene patterns. The difficulties to match these comparisons could be explained by the fact that TGFbeta may act through Smad- and non Smad-dependent signaling (for a review see for instance Derynck and Zhang. Smad-dependent and Smad-independent pathways in TGF-beta family signalling. Nature. 2003 Oct 9; 425(6958): 577-84). In addition, TGF-beta receptors different from ALK1 and ALK5 may also be present in the endothelial cells used in this study and a distinct stoichiometry of the receptors could account for the differences observed. All these limitations should be discussed.
3. The statistical test to determine the differential gene expression must be clearly explained in Methods and indicated in the specific table/figure. The “Measurement data and analysis” item in the Methods section (page 7) is insufficient.
4. The manuscript contains numerous results that may be of interest to investigators in the field, but the authors must improve the overall presentation of the data. To facilitate interpretation of results, it would be helpful to include a final schematic figure showing the overlapping gene expression patterns among the different endothelial cells. This figure should include only those genes whose expression is significantly altered.
5. Background, page 4, 1st paragraph. “Endoglin binds TGF-beta1 and -beta3 isoforms that requires presence of the TGF-beta type II receptor [16, 17]”. The original references must be included. For example, that endoglin binds TGF-beta1 and -beta3 isoforms was first described by Cheifetz et al. (J. Biol. Chem. 1992 Sep 25; 267(27): 19027-30). In addition, the fact that endoglin requires the presence of the TGF-beta type II receptor to bind ligand was first reported by Letamendia et al. (J. Biol. Chem. 1998 Dec 4; 273(49): 33011-9). Reference #16 could be deleted.
6. Background, page 5, 1st paragraph. “We believe this is the first time that a comparative gene-array and QR-PCR approach to the transcriptome for TGF-beta/ALK1 signalling has been attempted...” Since Ota et al. (Reference #37) and Lamouille et al. (Reference #37) have already reported similar gene expression analysis of ALK1-regulated genes, this statement should be softened or deleted.

7. Results section. The headings should be more consistent and organized. For example, there is no need for the first two separate items (“ALK1-induced genes in HMEC-1 cells” and “Gene expression in HMEC-1 cells 4 hours post AdALK1QD infection”).

8. Results, page 16, 2nd paragraph. “We were wondering if ALK1 signalling needs co-expression of endoglin.....” The authors give for granted the involvement of endoglin in the ALK1 signaling pathway. The Background section should include the findings that endoglin physically interacts and functionally collaborates with ALK1 (Lebrin et al. EMBO J. 2004 Oct 13; 23(20): 4018-28; Blanco et al. J. Cell Physiol. 2005 Aug; 204(2): 574-84).

9. The gene expression analyses were carried out with the TGF-beta1 isoform (table 1, table 6, and table 7), while reporter assays (Fig. 2) were carried out using both TGF-beta1 and TGF-beta3. However, no explanation is provided for this inconsistency. Why do the authors use TGF-beta3 for some experiments and TGF-beta1 for others?

10. The authors try to relate their findings with the Rendu vascular dysplasia. However, they should address the limitations of the endothelial cell culture model used versus an animal model of ALK1+/- mice that, according to literature, reproduces the HHT phenotype. Ideally, the altered gene expression pattern observed in endothelial cells should be confirmed in these HHT mice.

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. Background, page 3, 2nd paragraph, line 9. “transcriptionfactors” should be “transcription factors”
2. Methods, page 5. “(100U/ml),and” should be “(100U/ml), and”
3. Throughout Methods. The use of Liter/liter, milliliter/ml should be unified.
4. Methods, page 7, last paragraph, line -2. “manufacture’s instruction” should be “manufacturer’s instruction”
5. Methods, page 8, 2nd paragraph, the use of min./min, sec./sec should be unified.
6. Methods, page 8, 2nd paragraph, line 5. “spicific” should be “specific”
7. Methods, page 8, 2nd paragraph, line 7. “seperated” should be “separated”
8. Methods, page 8, last paragraph, line 1. “diluted 1in 50” should be “diluted 1 in 50”
9. Methods, page 8, last paragraph, line 3. “[Applied Biosystems]” should be “(Applied Biosystems)”
10. Methods, page 8, last paragraph, line 2. “[Eurogentec]” should be “(Eurogentec)”
11. Methods, page 9, second paragraph, line 5. “manufacture’s instruction” should be “manufacturer’s instruction”
12. Results, page 12, last paragraph. “LCLC cells.” Can be deleted
13. Results, page 14, line 2. “constuitive active ALK1” should be “constitutively active ALK1”
14. Results, page 14, 3rd paragraph, last line. “expresseion” should be “expression”
15. Results, page 15, heading. “avtive” should be “active”
16. Results, page 15, 3rd paragraph. “did not resulted” should be “did not result”
17. Results, page 15, last paragraph. “Next, we tested if TGF-b1 induced (SBE)4 activity can be restored by...” Do the authors mean “Next, we tested if TGF-b1 unresponsiveness to (SBE)4 reporter can be restored by...” ? This sentence should be rephrased.
18. Discussion, page 17, second paragraph, line 7 from bottom. “collage” should be “collagen”
19. Figure 1 legend, last line. “quantative” should be “quantitative”. Also, panels A and B in figure are not explained.
20. Figure 2 legend, lines 3 and 4. “....reporter constructs as indicated in A. – F. either alone or in combination with different receptor constructs as indicated.” Could be replaced by “.....reporter constructs either alone or in combination with different receptor constructs as indicated.”
21. Figure 2 legend, last line. “(see M&M)” should be “(see Methods)”
22. Table 2 heading. “List of genes analysed by QR-PCR and the ABI Assay on Demand used.” As such it is unclear and should be modified.
23. Labeling of histograms from Fig. 2 is confusing
24. Table 3, Signalling molecules. “Interleukine 8” should be “Interleukin 8”
25. An extra copy of figures 1-3 is included at the end of the manuscript
26. Methods, page 7. Some headings are underlined and some are in bold. Are the underlined ones subitems? Please unify

Discretionary Revisions (which the author can choose to ignore)
1. The description and source of the expression plasmids encoding ALK1, ALK5 and TbetaRII are missing.
2. The source of the TGFbeta isoforms 1 and 3 is not indicated

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: Needs some language corrections before being published

Statistical review: Yes

Declaration of competing interests:
I declare that I have no competing interests