Reviewer’s report

Title: The tumour-promoting receptor tyrosine kinase, EphB4, regulates expression of integrin beta8 in prostate cancer cells

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Reviewer: Paul Park

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In this paper, the authors employ transient knockdown of EPHB4 along with microarray gene expression analysis and functional assays to identify genes that may have a role in prostate cancer migration and invasion. Using these techniques, they determined that the integrin ITGB8 was significantly de-regulated in response to changing EPHB4 levels in prostate cancer cells, that they are concomitantly expressed in prostate cancer and that both are highly expressed in prostatic intraepithelial neoplasia. Based on these findings, the authors suggest that EPHB4 and ITGB8 could be involved in the onset of prostate cancer and targeting these two proteins synergistically may impact PCa progression.

The key contributions of this paper are confirmation of previously published functional link between EPHB4 and ITGs. These established links support and buttress the results which are not clearly reported, and the presented findings not very compelling at times. Some major points remain unclear, and need to be re-addressed by the authors.

Major compulsory revisions:

1. In their discussion of gene ontology screening following EPHB4 down-regulation in LNCap, the authors claim there were three integrin molecules (ITGB8, ITGA3 and ITGA10) that showed de-regulation from their microarray analysis. To confirm this data, they proceeded with assessing the expression levels using real-time PCR on a different set of LNCaP transfectants. In the second set of transfectants, however, ITGA3 was unchanged. This brings into question whether Figure 1B represents an average of the changes observed for both transfectants following down-regulation, and whether this explains the large SD observed for ITGA3. This point needs to be elaborated, especially since the statement made in the second paragraph of Results is not consistent with that made in the first paragraph of Discussion.

2. In correlating ITGB8 and EPHB4 expression levels, the authors suggest that the upregulation of both genes in prostatic intraepithelial neoplasia relative to benign samples, and their down-regulation in metastatic samples, may indicate that the expression level of EPHB4 and ITGB8 may be progressively lowered with disease advancement. To investigate this, the authors examine the expression in different prostate cancer cells representing different stages of the disease. In order for the proposed relationship to hold true, one would expect to
see a much lower expression of ITGB8 in the PC-3 cell line, which was initiated from a bone metastasis of a grade IV prostatic adenocarcinoma. PC-3 cells have high metastatic potential compared to DU145 cells which have a moderate metastatic potential and to LNCaP cells which have low metastatic potential. Therefore, one would expect LNCaP to have the highest expression level of ITGB8 expression, followed by DU145 and PC-3, with the latter having the lowest levels as it is most closely associated with disease advancement.

3. In general, the data presented in the Figures 1-3 is difficult to interpret, given the poor description of the statistical analysis in the Methods and figure captions. In Figures 1B and 1D, the calculation mRNA in Y axis should be described: “relative mRNA expression/control/GAPDH” does not yield a meaningful comparison, whereas “relative mRNA expression/GAPDH/control” would be an appropriate measure.

Minor Essential Revisions:
1. Due to the poor quality of the image showing western immunoblotting for EPHB4 knockdown in LnCaP, the degree of down-regulation is difficult to discern, and therefore, not very compelling.
2. Standardized nomenclature of genes and proteins, as assigned by the HGNC, should be adhered to more rigorously to avoid ambiguous scientific exchange.

**Level of interest:** An article of limited interest

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests.