**Reviewer's report**

**Title:** Investigation of the expression of the EphB4 receptor tyrosine kinase in prostate carcinoma

**Version:** 1  **Date:** 27 May 2005

**Reviewer:** MS Kinch

**Reviewer's report:**

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

This is an interesting manuscript that documents that overexpression of a potentially interesting and relevant target for cancer. The most interesting and convincing findings relate to the overexpression of EphB4 in clinical specimens of prostate cancer and it is strongly recommended that these studies be emphasized in a revised form of the present manuscript.

The primary issues revolve around unsupported conclusions. For example, the differences in expression between different cell lines (LNCaP, DU145, PC3) appear insignificant, yet emphasis is placed upon this in the second paragraph of the Discussion.

Likewise, the difference in subcellular localization between cell lines is unconvincing. Greater emphasis should be placed upon the presentation of data that would support this conclusion.

To this end, a conclusion (from the Discussion) is that differences in subcellular localization would reflect differential tyrosine phosphorylation of EphB4. Therefore, it seems appropriate for the investigators to ask whether the perceived differences in subcellular localization directly relate to the levels of EphB4 tyrosine phosphorylation.

The most important (and far-reaching) outcome of the present study is that high levels of EphB4 are found in clinical specimens of prostate cancer. Therefore, greater emphasis should be placed upon both the analysis and implications of this finding. For example, the authors accurately point out that prostate cancer is often multi-focal. Therefore, the specimens under investigation likely have different grades of the diseases (including PIN) and it would be interesting to determine if EphB4 levels correlate with histologic grade.

It is important that the authors specify whether the results were reviewed by a certified pathologist. This is particularly important if the studies were to presented in the context of differential staining of more aggressive forms of the disease (see the paragraph above).

Table 3 (EphB4 localisation) is confusing. If this is to be a major point of this manuscript, then representative photos that detail the differences between the three categories should be provided. Also, the Discussion should be amended to explain the potential implications of this finding.

Finally, it is strongly recommended that the Discussion be revised. At present, it reads as a simple recapitulation of the Results. It would be more informative were this to be revised to emphasize greater evaluation how the present findings relate to the investigation of EphB4 in other cancers and/or how EphB4 relates to other markers and targets in prostate cancer.

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Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the
There appears to be typographical error in the last sentence of the last paragraph of the Background.

With regards to the lower-mobility band in Figure 2, the authors seem to overreach the conclusion that this represents an alternatively phosphorylated form of EphB4. This could likewise represent an alternative splice or cross-reactivity with a related (or unrelated) antigen (particularly since these studies rely upon the use of a polyclonal sera).

It would be useful to update the IHC data (Table 2) to include averages of immunoreactivity.

It is recommended that β-catenin be removed as a loading marker since this molecule is known to be aberrantly regulated in PC3 cells.

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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'