Author's response to reviews

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

Authors:

Yen-Ching Lee (ken.lee@student.adelaide.edu.au)
Janeanne R Perren (jananne.perren@student.adelaide.edu.au)
Evelyn L Douglas (evelyn.douglas@adelaide.edu.au)
Michael P Raynor (mraynor@primus.com.au)
Maria A Bartley (ants75@hotmail.com)
Peter G Bardy (Peter.Bardy@imvs.sa.gov.au)
Sally-Anne Stephenson (sally-anne.stephenson@adelaide.edu.au)

Version: 2 Date: 9 August 2005

Author's response to reviews:

Dear Iratxe,

We have revised our manuscript entitled "Investigation of the expression of the EphB4 receptor tyrosine kinase in prostate carcinoma" by Lee et al to address the comments of the two reviewers. To address the revisions required by Prof. Sugimura, who commented on differences between our RT-PCR and western data and those of Xia et al recently published in Cancer Research, we have repeated our western analysis and used a more appropriate control to confirm equal loading. The new data has been included in Figure 3. Dr Kinch also requested the removal of b-catenin as a loading control and this has been done. We also used a positive control for this sample (EphB4 over-expressed in the MCF10A cell line) to identify which of the two immunoreactive bands is wild-type EphB4 and have included the possibility that the second band could be an alternative spliced, glycosylated or phosphorylated form. We use a monoclonal antibody (Zymed) for our Westerns so we do not believe that this is cross-reactivity with an unrelated antigen (which is possible with a polyclonal sera).

We also repeated the real-time PCR using RNA extracted from cells grown to different confluencies with several repeats and are confident that our normalisation data is accurate and shows little difference in gene expression in these cell lines as we previously reported. The extra data was described but not shown as a Figure. A possible reason for differences with Xia et al is their use of B-actin for normalisation, a gene which is highly expressed relative to EphB4.

Prof. Sugimura also comments that the number of cases we report are small. We consider this to be a pilot study and the results gained warrant further examination of EphB4 in prostate cancer. Xia et al (2005) used tissue arrays to examine EphB4 expression in prostate cancer and did not draw any conclusions regarding level of expression and stage of disease. Because the tissue that we used contained both normal and tumour foci our results suggest a trend towards increased EphB4 expression in higher grade disease. We have included more of the immunohistochemical data (Figures 5 and 6) to demonstrate this and have sought advice from a certified pathologist. As pointed out by our second reviewer Dr Kinch, this is an important conclusion from our results. We have removed Tables 2 and 3 as they were considered confusing and much of the data is now presented in Figures 5 and 6 anyway.

We have removed the emphasis in the discussion regarding the subcellular localisation of EphB4 in the cell lines but have not changed the figure. We are planning further studies to address this but will report these at a later date.

Finally we have substantially revised the discussion to emphasize how these results relate to the investigation of EphB4 in cancer.

Thankyou for your consideration and please let me know if you require any further information,

Dr Sally-Anne Stephenson
Senior Research Fellow
Cancer Council of South Australia
Department of Haematology/Oncology
The Queen Elizabeth Hospital