Reviewer's report

Title: Analysis of gene expression in prostate cancer epithelial and interstitial stromal cells using laser capture microdissection

Version: 1 Date: 28 September 2009

Reviewer: Geert Van Leenders

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Interaction of prostate stroma with adjacent benign and malignant glands is of seminal importance for normal prostate function, and tumour biology. While many proteins are differentially expressed within the stromal and epithelial compartments in the prostate, relatively little is known about the molecular pathways regulating stromal-epithelial interaction. In this manuscript Reese and colleagues use an elegant micro-array methodology after specifically selecting stroma and malignant glands form human prostate cancer by laser capture microdissection.

Major compulsory comments:

While gene-expression analysis on pure cell populations represents a powerful tool to explore important pathways for stromal-epithelial pathways in both normal and malignant prostate tissue, the current manuscript is rather descriptive and does not clearly state a rationale goal for its stromal and epithelial analysis. For instance it would have been very interesting to correlate expression patterns of 'tumour-associated' stroma with normal stroma.

After the thorough analysis of the gene-expression data, the authors validate 6-10 interesting genes on a set of tissue specimens and cell lines. The validation strategy is however somewhat remarkable. First, the authors analyze 6 genes on LCM purified cell populations. Currently, it seems that this validation set is not independent, but rather equals the test-set. For proper validation, however, an independent tissue set is required. Second, the authors validate their gene set in a group of ten non-purified paired frozen tumour and non-neoplastic tissues, which are generally constituted of a large mix of cell types. While a very elegant way of gene discovery is used in this study, interpretation of the validation data is limited due to the contamination of various cell types. The validation of the gene-expression profile in cell lines is interesting for future function studies, but has very limited value for representability as validation set obtained from patients' tissues. Also, one cannot draw strong conclusions comparing normal and cancer cells since only one benign cell line was included.

Minor essential comments:

Page 3, first paragraph: the text can be formulated more properly. For instance, "prostate adenocarcinoma is characterized by invasion of luminal spaces and ..."
Areas of the slides observed to have the most abundant cell of interest were identified. It is not clear whether the stromal cells are derived from the same area as the malignant cells. Often stroma contains inflammatory cells; was the presence of inflammation accounted for?

As expected, the stromal genes are more highly expressed in non-neoplastic tissue than in paired tumor tissue. Why is this expected? Was this because normal tissue obtains more stromal cells? What was the tissue composition in respect of stromal cells, inflammatory cells, pre-existent glands and cancer glands?

Page 9, middle: what is the rationale of analyzing the proteins separately in T2 and T3 tumours?

Page 9, middle: what is the meaning of cytoplasmic WT1 staining? Is this background or really a functional state of the protein?

Figure 3: it is not entirely clear what is represented in this figure.

Figure 5: the non-neoplastic and BPH tissues look rather similar. How are both defined in this study?

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

**Quality of written English:** Acceptable

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**

I declare that I have no competing interests