Reviewer's report

Title: Differences in gene expression in prostate cancer, normal appearing prostate tissue adjacent to cancer and prostate tissue from cancer free organ donors

Version: 4 Date: 11 February 2005

Reviewer: Remi Houlgatte

Reviewer's report:

Major Compulsory Revisions:

1) The normal and donor populations have a very different age distribution. 7% of tumour cases are under 50, whereas 60% are under 40 for the normal donors. This point is discussed in a short paragraph of page 7, but no data are shown. This is an important issue, and some data should be provided to the reader. This could be obtained either by
   - Classification methods: Does the Donor above 40 closer to adjacent samples than those below?
   - ANOVA: What is the impact of age in variance analysis?
   - Prediction methods: If the genes differentially expressed between donor below 40 and adjacent tissue, are used, in which class, donor above 40 are predicted?
   Or another appropriate method ...

2) Furthermore, normal donor samples and tumour and adjacent tissues are collected in a quite different fashion. All these factors may cause differences in gene expression.
   For example, long-term bathing in Ringer solution of donor samples may lead to a decrease in blood cells in the sample, and an increase in early-response genes like Fos and Jun (see Perou et al. 2000 Nature 406: 747). This phenomenon may account for the observed up-regulation of Fos (4 different probesets), Jun (v, B, D; 5 genes), EGR1, Immediate Early protein ..., and down-regulation of immune response (4 genes), Blood clotting (1 gene), Plasma protein (1 gene), Blood group antigen (1 gene) genes in Donor vs Tumour comparison.
   A total of 18% (11/50 + 7/51) of genes could be affected by this phenomena. This is higher than the effect on 1% of the genes, cited by the authors on page 9. Discussion on this point must be improved.

3) These controversial points (Major points 1 & 2) make necessary that the author make their data publicly available. This should be done according to MIAME guidelines

Minor Essential Revisions:

4) Page 3 Method Section: The distribution of age is missing for adjacent normal samples.

5) Page 4 bottom "Therefore, in the interests of clarity, we will focus on the MAS 5.0 results in the remainder this paper." This sentence is not correct: figure 1 shows dCHIP plots.

6) Page 8 Top: Differential expression of muscular genes is a difference in tissue composition (absence of muscular cells in tumour samples). This phenomenon has been observed in Alon et al 1999 PNAS 96:6745. It is not related to oncogenesis.

7) Page 9 Paragraph 2: “Our donors did not have prostate cancer or PIN identified
... "PIN? Prostatic intraepithelial neoplasia?"

**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 limited interest

**Quality of written English:** Acceptable

**Statistical review:** No

**Declaration of competing interests:**

I declare that I have no competing interests.