Reviewer's report

Title: Microarray Analysis in Clinical Oncology: Pre-clinical Optimization Using Needle Core Biopsies From Xenograft Tumors

Version: 1 Date: 3 March 2004

Reviewer: Laszlo G Puska

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General
The authors are dealing with a very important aspect of present microarray analysis strategies: they confirmed that tumor biopsies can be reliable used in microarray experiments by using linear amplification protocol. The in vivo model systems presented by the authors are good test systems to analyze the gene expression profiles generated from needle core biopsies. They showed good correlation obtained by biopsy samples to the gene expression profile of the whole tumor. They tested four variables: minimal amount of starting RNA, the effects of linear amplification, labeling protocol and the gathering of the sample (snap frozen or in RNAlater). It is also very important that they described that RNalater preserved samples are as good as frozen ones in microarray studies, i.e. “RNA later” reagent does not influence the results obtained from microarray hybridization.

The methods are appropriate and well described, and are sufficient details provided to replicate the work.
The discussion and conclusions well balanced and adequately supported by the data, except that was mentioned in the Minor revision part.
The manuscript is very well written and organized, the figures and tables can be understood well and useful in supporting of the statements.

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)
NO

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

In the Conclusion section the authors conclude that „These data suggest that needle core biopsies can be used as reliable tissue samples for tumor microarray analysis after linear amplification and indirect labeling of the starting RNA.”, however, later in text it turned out that in reproducibility and reliability there was no difference between direct and indirect labeling protocols. Therefore it is a little bit confusing that in the Conclusion they mention and suggest only the indirect labeling approach. Please clarify or include both methods in the conclusion.

Some spelling mistakes:
In the abstract „RNA (0.3-15 ?g).” instead of RNA (0.3-15 ?g). The same typing mistakes can be found several times in the Materials and Methods section and over the manuscript. Also instead of „?I”, „ul” can be found in several places. All the concentrations should be checked and corrected over the manuscript.

„scatter plots” instead of „scatter blots” in the Result in page 11.

On page 18, „Sotiriou et al." instead of „Sotiriou et al".
Discretionary Revisions (which the author can choose to ignore)

What would be the concordance level among the same experiments if a dye-swop experiment is performed? Although this work focuses on several factors, like sample preparation, use of RNase blocking reagent, biopsies, etc. a dye-swop experiment would define more accurately the concordance level of the replicate experiments. Although other studies have been performed to determine the effects of the incorporation of different dyes, in this work it would have confirmed the presented data more reliable, i.e. could provide a rough estimate on the real changes detected in the case of different protocols. However, the authors presented a series of experiments on other (and more important) variables, which are well carried out and appropriate for confirming their findings.

Which journal?: Appropriate or potentially appropriate for BMC Medicine: an article of outstanding merit and interest in its field

What next?: Accept for publication in BMC Medicine after minor essential revisions

Quality of written English: Acceptable

Statistical review: No

Declaration of competing interests:

None