Title: Assessment of the role of transcript for GATA-4 as a marker of unfavorable outcome in human adrenocortical neoplasms

Authors:

   Angela S Barbosa (angela.barbosa@hcnet.usp.br)
   Luciano R Giacaglia (lucianogiacaglia@ig.com.br)
   Regina M Martin (reginamm@usp.br)
   Prof Berenice B Mendonca (beremen@usp.br)
   Dr Chin J Lin (cjlin@bol.com.br)

Version: 2 Date: 9 Feb 2004

PDF covering letter
Dear members of the editorial board,

Please find enclosed a revised version for our manuscript # 1625451411217454 entitled “ASSESSMENT OF THE ROLE OF TRANSCRIPT FOR GATA-4 AS A MARKER OF UNFAVORABLE OUTCOME IN HUMAN ADRENOCORTICAL NEOPLASMS”. References quoted in this new version have been formatted using EndNote 6.0. In order to comply with the reviewers’ requests we have performed additional experiments which, in our opinion, confirmed our initial findings and strengthened our paper. As suggested by Dr Peri, we complemented our RT-PCR experiments with dot-blot hybridization which improved both sensitivity and specificity, and also allowed a quantitative comparison of relative expression of GATA-6, GATA-4 and LHR transcripts. Modifications in our manuscript were made accordingly to incorporate these new data. Since Dr Regina M. Martin participated substantially in these new experiments and manuscript revision she was included as a co-author of this paper. Listed below are changes we performed as well as our reply to the reviewers’ comments.

Minor changes in the abstract to accommodate the inclusion of dot-blot and new experimental results.

In “Methods”:

A more detailed explanation on how patients were categorized into nonmetastasizing and metastasising/recurrent group was provided (page 6, line 7).
More information concerning patient 22 and comments on the revision of histopathological data was added (page 6, line 12, 15).
Procedure for dot-blot hybridization and statistical analysis were included (page 8 and 9)

In “Results”:

Weiss scores were included in Table 1.
The previous version of Table 2 was removed and replaced by a new version containing densitometric data of GATA-6, GATA-4, and LHR autoradiograms.
All RT-PCR gels were consolidated into one single figure. This same figure also presents dot-blot images (Figure 1 a to e).
The old Figure 2 was replaced with a new one which now shows charts correlating GATA-4 expression with LHR and GATA-6.
Comments on the influence of age and hormonal secretion on GATA expression in our specimens were deleted in this section and in “Discussion”.
Changes in our results due to dot-blot (Figure 1b, Figure 1c, and Figure 1d). Modifications in the “Results” section were added accordingly (pages 11 and 12). A new subsection in “Results” analyzing the usefulness of GATA and LHR transcripts in discriminating the clinical fate of adrenocortical tumors was included.

In “Discussion”:

Comments and conclusion regarding the usefulness of GATA-4 and LHR in prediction of prognosis were changed in accordance to our new results (pages 13 and 14).

Responses to specific comments from the reviewers:

Replies to Dr Wilson:

Minor Compulsory Revisions:

We agree that “prevalence” or “frequency” are more accurate words than “rates” to describe the number of positive samples. In accordance to new results incorporated into our paper, the sentence containing the inaccurate term “rates” was deleted.

Major Compulsory Revisions:

We included dot-blot hybridization. This method improved both sensitivity and specificity. Moreover it made possible a quantitative measurement of transcript accumulation. These new, quantitative, data allowed us to establish threshold values for GATA-4 and LHR which might, potentially, predict the clinical fate of patients harboring adrencocortical neoplasm.

Replies to Dr Peri:

As suggested in your general comments we adopted dot-blot as a complement to our non-quantitative RT-PCR. In fact, this resulted in improvement of both sensitivity and specificity. Quantitative data of transcript accumulation are presented in this revised version.

Minor Compulsory Revisions:

Autoradiograms of dot-blot hybridization were added to the new version of Figure 1 along with gel photographs. This should allow an unequivocal distinction between positive and negative samples. We have now corrected the missing data in Table 1.
**Major Compulsory Revisions:**

Histological classification of adrenal tumors into adenoma and carcinoma can be very difficult and not always reflects the true clinical fate of patients. As stated by some authors, the only definitive criteria for malignancy are distant metastasis and/or local invasion (Gicquel, C 2001, Aubert S 2002). Additionally, Weiss score system tend to be inaccurate in pediatric patients (Mendonca BB 1995, Sredni ST 2000). Therefore, we categorized our patients according to their clinical outcome. As requested, Weiss scores were added when available histopathological data were sufficient for their calculation. We also included a more detailed description on how our patients were grouped into NM and MR subsets.