Reviewer's report

Title: QPRT: a potential marker for follicular thyroid carcinoma including minimal invasive variant. A gene expression, RNA and immunohistochemical study.

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Reviewer: Juan Pablo Rodrigo

Reviewer's report:

In this work the authors search for new markers that could help in the diagnosis of FTC. They have identified QPRT protein as a potential immunohistochemical marker for this purpose. However, the sensitivity (65%) and specificity (73%) of this marker are low, as compared with other markers described in the literature. Especially, the low sensitivity (35% of malignant cases were not detected) makes the utility of this marker doubtful. In addition, the authors indicate the difficult differential diagnosis between FTA and FTC in histological sections, when the main problem is to establish the correct diagnosis of a thyroid nodule. Although fine needle aspiration (FNA) cytology is very useful in identifying benign lesions and papillary carcinoma, the morphologic distinction of hyperplastic adenomatous nodules, well-differentiated follicular carcinomas, and follicular variants of papillary carcinomas is difficult, even for experienced cytologists. As a consequence, most patients with these sorts of lesions are referred for surgery for diagnostic purposes. In such cases as these, molecular analysis offers the potential of acting as an adjunct that might serve to improve the accuracy of FNA. Then these markers should be tested in samples obtained by FNA instead than in histological samples. Based in these considerations, the article has some interest but the clinical relevance is low.

- Major Compulsory Revisions

Methods:
- The authors must indicate which positive and negative controls (if any) were used in immunohistochemical analysis. The use of these controls is recommended.
- How was the staining pattern? Homogeneous? Patched?... and how were the positive cells counted?

Results:
- The data presented in table 2 and in figure 2A are the same. Consequently, figure 2A could be eliminated.
- If the samples for immunohistochemistry, western blotting and qRT-PCR were obtained from the same cases, it would be interesting to know if there was an agreement among the results obtained with the different methods.

Discussion
- The authors stated that “QPRT turned out to be discriminating between FTA and FTC”, however this affirmation is not sustained by the data presented in the article, because the high rate of false positive (26.5%), and especially, false negative cases (29.4%) suggest that QPRT could help in the diagnosis of FTC, but does not identify malignancy in all the cases. This must be emphasized in the discussion, moreover when other markers have shown a better accuracy.

- Important references about molecular markers used in the diagnosis of follicular neoplasms are lacking, especially about galectin-3 the most widely used marker to distinguish between FTA and FTC, for example: Bartolazzi A et al, Lancet Oncol 2008;9(6):508-10; Sanabria A et al, Head Neck 2007;29:1046-94. In these studies, galectin-3 showed a higher sensitivity and specificity than in those cited in the article.

- Finally, the limitations of the study (sensitivity of the marker, validation on FNA cytology) should be clearly specified in the conclusions.

- Minor essential revisions
- Tables 1 and 3 are unavailable in the PDF file
- Discretionary Revisions
Methods:
- Indication of the primers used in RT-PCR is recommended.

Results
- The positive and negative predictive values should be included. They could be calculated from table 2, but they are relevant for the purposes of the article and should be mentioned.

Discussion:
- The authors indicate that the differences in gene expression between the different studies could be explained by the platform used. This means that results are more platform-dependent than tissue-dependent, questioning the validity of this approach. To be scientifically sound is to be expected that if the same genes are present, although different platforms were used, the studies must show similar results.

**Level of interest:** An article whose findings are important to those with closely related research interests

**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