Reviewer's report

**Title:** Reduced expression of N-Myc downstream-regulated gene 2 in human thyroid cancer

**Version:** 2  **Date:** 12 April 2008

**Reviewer:** Theodoros Foukakis

**Reviewer's report:**

In this study of Zhao and colleagues the role of NDRG2, a gene regulated by N-Myc, is investigated in thyroid tumors. The authors use both a commercially available tissue microarray and primary tumors collected at their institution, and examine the expression of NDRG2 with a number of methods, at the mRNA and the protein level. The study is potentially interesting, but there is a number of major points that need to be improved and clarified.

**Major compulsory revisions:**

1. The first paragraph on page 4 (background) needs to be revised. The statement "Presently, our understanding of the development of thyroid tumors is poor" does not reflect the current status of research in the field, and the 2 citations provided are irrelevant. There is a recent review article in Nature Reviews Cancer that summarizes the enormous amount of progress made during the latest decades and that could serve as a reference. Moreover, the reader would expect background information about the role of Myc in thyroid and the reason why NDRG2 was studied in this tumor type. The last part of the "background" can be omitted as it merely repeats extensively the findings of the study.

2. I am sceptical about the commercial tissue microarray used in the study. Are there any previous publications using this array? Any quality controls? Very limited information is provided in Table 1, that raises some concerns: Are the "normal" samples taken from patients with thyroid cancer or other diseases e.g. thyroiditis? Surprisingly, the mean age of patients with undifferentiated carcinomas here is 43 years, while it is known that this is a disease of the elderly (>70 years).

3. The method for quantitative PCR is unclear. Which chemistry was used (SYBR green?)? Please state. Was there any control for the quality of mRNA? Was the endogenous control gene (GAPDH) consistent throughout the samples?

4. Why are the results of immunohistochemistry not shown for the primary tumors? Equally, the PCR data are shown only for groups, but not for every individual case. I think it is essential to provide a table with the results of all methods (PCR, Western, Immunohistochemistry) for all cases. How big was the variation between cases? Were RNA and protein levels correlated? Were the levels of Myc inversely correlated with those of NDRG2?
5. As very limited raw data is provided (comment 4) it is difficult to assess the importance of the findings described. However, the discussion and conclusions should be revised as there are several points that are not supported by the results (for example "Our results showed significantly reduced expression of Ndrag2 in thyroid cancers, indicating the involvement of NDRG2 in the process of thyroid carcinoma formation").

6. No functional studies are presented here, so the statement "Next, we investigated the function of the transcriptional repression of Myc on human NDRG2 in thyroid cancer" in the discussion is incorrect.

7. The writing is poor, in terms of both the language and the structure, and should be extensively revised.

Minor Essential Revisions

1. The terms "tumor" and "cancer" seem to be used interchangeably throughout the text by the authors which is confusing, as "tumor" can also refer to benign neoplasms.

2. In table 1, explain what age and the +/- refer to. Is it in years and SD? Please clarify.

3. In table 2 explain the abbreviations PTC, FTC etc.

4. In table 2, I think p in pTNM stands for "pathologic" not "primary"

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

**Quality of written English:** Not suitable for publication unless extensively edited

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests