Author's response to reviews

Title: Mitochondrial DNA Mutations in Oxyphilic and Chief Cell Parathyroid Adenomas

Authors:

Jessica Costa-Guda (costa@nso2.uchc.edu)
Takehiko Tokura (ttokura@med.kawasaki-m.ac.jp)
Sanford I Roth (siroth@northwestern.edu)
Andrew Arnold (molecularmedicine@uchc.edu)

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Author's response to reviews: see over
We appreciate the reviewers’ attention to our manuscript. We have responded to their suggestions and comments as detailed below, and believe the manuscript is considerably improved as a result.

Reviewer 1

1. “Total 393 homoplasmic sequence variants were identified. Among them, only 11 were somatic mutations. Although somatic mutations are generally pathogenic, the remaining germline sequence variants may also contribute to disease development. The nature of these mutations should be presented in a table, esp. those 32 variants predicted to affect rRNA or tRNA.”

We have now included a table (Table 1) listing the novel germline sequence variants identified in this study.

2. “How the authors explain such a high frequency of germline sequence variants present in both tumor tissues and peripheral blood DNA? Are they mutations?”

The finding of a number of sequence variants is common in studies of mitochondrial sequencing, especially when the entire mitochondrial genome is examined, as in our study. The number of sequence variants identified in the samples not associated with parathyroid disease was equivalent to those found in parathyroid hyperplasias and adenomas. While we cannot exclude the contribution of germline sequence variants as predisposition alleles, there is no compelling reason to suspect that they contribute directly to parathyroid tumorigenesis.

3. 33.3% (6/18) chief cell adenoma had somatic mtDNA mutations as compared to 75% (9/12) oxyphil adenoma. I am wondering whether those germline sequence variants also had the same distribution. If they do, these variants may be mutations.

We identified sequence variants in every sample in our study, including normal parathyroid glands. There was no difference in distribution of sequence variants between chief cell and oxyphil cell adenomas. This is now explicitly stated in the manuscript on page 8.

4. mtDNA transitional mutations are most often detected in tumor samples as reported in the literature. The interesting part of this study is that high frequency of somatic insertion/deletion was found in tumor samples (29.62%, 8/27 mutations): 18.51% (5/27) in oxyphil adenoma and 11.11% in chief cell adenoma. Was insertion/deletion ever found in those variants?

Insertion/deletions were rare, but not entirely absent as germline sequence variants. All of the insertions/deletions identified as germline sequence variants were located in non-coding regions, suggesting that these variants likely have little, if any pathogenetic significance. This is now explicitly stated in the manuscript on page 7.
The authors should include a figure showing those mutations, insertion or deletions.

A figure (Figure 2) showing the locations of the somatic mutations identified in this study has now been included.
Reviewer 2

The authors state in the Abstract that there was no clustering of sequence variants in the hyperplastic gland; this statement should be clarified (was there clustering in neoplastic glands? what were the results in the different types of lesions?).

**We thank the reviewer for pointing this out. We did not observe clustering of sequence variants in any of the samples. This statement has been clarified accordingly.**

The paper would benefit from a better description of the data namely a better description of the sequence variants regarding their type and localization within the genes of the MRC and their distribution among the different types of lesions.

**We thank the reviewer for this suggestion. We have now included a more detailed description of the data (page 7, paragraph 1 and Table 1).**

It is also very important to know the prevalence of the so-called large deletion of mitochondrial DNA which has been considered the hallmark of oxyphil cells in thyroid lesions, as well as in the oxyphil cells of their organs (Máximo et al Am J Pathol 160:1857-1865,2002; Sobrinho Simões et al Int J Surg Pathol 13:29-35, 2005). Were there mtDNA deletions? If the answer is yes, were there differences between oxyphil cells and chief cell adenomas (I would expect the answer is yes).

**We thank the reviewer for noting this important issue. The presence or absence of the common deletion of mtDNA was beyond the scope of this manuscript, but we may address this matter in the future.**

It would also be very important to compare the somatic mutations found in the present study with those reported in other studies (Yeh et al Oncogene 19:2060-2066, 2000; Máximo et al Máximo et al Am J Pathol 160:1857-1865, 2002) Finally, it would be important to discuss in depth the differences between oxyphil and chief cells adenomas, namely using the exiting literature on the alterations found in Hürthle cell lesions of the thyroid. I do not understand why the authors do not mention most of the papers on the molecular studies in Hürthle thyroid tumours. The comparison using the Fisher exact test (see below) appears to be too superficial. (Are as the observed differences enough to distinguish chief cells and oncotic cells?).

**We agree that it is important to compare our results to those of other studies; however, very often differences in study design (specifically, whether the full or only a partial sequence of the mitochondrial genome was performed) prohibit the direct comparison of our results with others. We have included a more detailed review of relevant studies of related oxyphilic/oncocytic tumors in the discussion (page 10, paragraph 2), including the findings by Gasparre, et al (PNAS, 2007), which were published after submission of our manuscript.**
The paper also needs factual corrections:
1. Abstract – Mutations in: 9 of 12 oxyphilic adenoma and 6 of 12 chief cell adenomas(?)
Methods – Cases 19 chief cell adenomas and 11 oxyphil adenomas(?)
Results – Mutations in 6 of 18 chief cell adenomas and 9 of 18 oxyphil adenomas
2. Fischer exact test “gave” a p value of 0.2 in page 8 of the Results and a p value of 0.02 in page 10 of the

We thank the reviewer for noting these factual errors, which we have now corrected within the manuscript.