Reviewer's report

Title: A mutation screening of oncogenes and nuclear encoded mitochondrial complex I genes in oncocytic thyroid tumors

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Reviewer: Barbara Kofler

Reviewer's report:

Evangelisti et al. report in thyroid oncocytomas on genetic alterations of nuclear encoded complex I genes and on genes typically altered in thyroid cancers. As oncocytic thyroid cancers have not been investigated regarding these genetic changes the manuscript sheds light on the genetics of these rare tumors. The most interesting finding was the occurrence of TP53 mutations in 3 out of 45 cases and of PAX8/PPARg rearrangement in 5 out of 10 investigated samples.

Major Compulsory Revisions
A detailed supplementary table has to be prepared listing all samples grouped according to subtypes (hyperplastic oncocytic thyroid nodules, thyroid oncocytic adenoma, carcinoma, thyroid follicular adenoma) and detected genetic changes (nuclear and mtDNA), histology, age of patients and gender.

Discussion: In line 314 the authors claim that the expression of the chimeric protein PPFP did not show any significant correlation with the presence of mtDNA mutations. However, the reviewer could not find any data on the expression of the chimeric protein in the methods and results section. The authors need to include the data and method.

As the frequency of TP53 mutations in the samples is very low (3 out of 45) and the incidence of mtDNA mutations is about 50% in the samples the co-occurrence of mtDNA mutations and TP53 mutations in two cases are more likely a coincidence, unless statistical analysis would indicate a clear cut correlation. Therefore, the speculation on the impact of TP53 mutations on the occurrence of the oncocytic phenotype is more than questionable and therefore, the emphasis given to this correlation in the abstract should be omitted and in the discussion should be even more speculative.

The message of the last sentence of the discussion is not clear the reviewer.

Conclusion: It is not obvious from the results that a significant co-occurrences of genetic effects have been found in the oncocytic tumors. This might get clearer once a table including all cases and genetic alterations is included.

Minor Essential Revisions
The exact method of DNA/RNA extraction should be given, as the extraction of high quality RNA is difficult from paraffin embedded tissues. This should include
the amount of tissue used for the extraction as well as the quality measures taken.

In the text the authors indicate TP53 mutations in 3 cases but in table 3 only two cases with frameshifts are mentioned. The authors need to include the missense mutation in the table.

Age range and gender should be given of the sample subgroups.

The full name of genes/protein should be given of all abbreviations used when first mentioned (e.g. RET, PCT, PAX8, PPARg)

Methods:
Line 149: change to „.....was performed“
As no reference is given for the methods of BRAFp600V>E and RAS codon 61 mutations it is necessary that primer sequences for PCR amplification are given.

Line 286: Oncocityc should be Oncocytic
Line 292: Include a space after the end of the sentence.

Discretionary Revisions
The number of samples analyzed should be indicated in the abstract.

Line 103: change the time to „....cells originate“. 
Line 125, 130, 285: The authors should also refer to some publications reporting primary data on mtDNA mutations in different oncocytomas and the functional impairment of complex I (eg. Hum Mol Genet. 2008 Apr 1;17(7):986-95)

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**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