Reviewer's report

Title: Somatic VHL gene alterations in MEN2-associated medullary thyroid carcinoma

Version: 2  Date: 30 March 2006

Reviewer: Attila Patocs

Reviewer's report:

General comments: Germline mutations and/or deletions of VHL gene are associated with von Hippel-Lindau syndrome, as well as, VHL gene alterations play a role in the pathogenesis of apparently sporadic phaeochromocytomas. MEN2 is a familial cancer syndrome, which is caused by germline mutations of RET protooncogene. Phaeochromocytomas may occur in both MEN2 and von Hippel-Lindau syndrome. Somatic VHL gene mutations are implicated in the pathogenesis of sporadic phaeochromocytomas. The authors hypothesized that somatic VHL gene alterations might have a role in the pathogenesis of MEN2-related medullary thyroid cancer (MTC).

Study design: In this study the authors present the molecular biology analysis of 6 MTCs and 1 C-cells hyperplasia obtained from 7 MEN2 patients with known RET mutations. They did allelic-imbalance analysis of RET and VHL genes as well as they did the mutation testing of VHL gene at somatic level.

Results and Conclusions: In 3 of 6 MTC’s the amplification of RET and LOH of VHL gene were proved using polymorphic markers. In the other 3 MTC’s and in 1 C-cell hyperplasia no genetic alterations of these genes were found. In one patient, in addition to LOH of VHL locus, one somatic mutation also was identified. The authors conclude that germline RET mutation is necessary for development of C-cell hyperplasia, that allelic imbalance between mutant and wild-type RET may set off tumorigenesis, and somatic VHL gene alterations may not play a major role in tumorigenesis of MEN2A-associated MTC.

Major compulsory revisions: The sample size used in this study is very small, and the results found may change after extending the investigation. This article is not suitable for publication as a full length Research article, because the data presented here represent just some preliminary results. The LOH of VHL locus and the somatic frameshift mutation found in MTC argue that the VHL gene might have effect in tumor progression of MEN2A-associated MTCs (3 of 6 MTCs have LOH). The methods used in this study are appropriate and well designed, the microsatellite markers used for LOH analysis are mapping the corresponding chromosomal regions, but the interpretation of the sequencing result of the specimen with frameshift mutation is not correct. The chromatogram shows the deletion in heterozygote form, but the authors reported that the same specimen has LOH of the VHL gene, but in that case the chromatogram should show just the mutant allele. The authors need to revise the analysis and the interpretations of result. The mutation analysis of exon 2 and exon 3 of VHL gene failed in 2 specimens because “technical problems” (Pages 5-6). The authors need to resolve this type of “technical problems”, but also is possible that those tumors may have polymorphisms or mutations on the priming sites of primers used for amplifications. Redesigning the primers may lead to the successful analysis. Also, it is possible that the quality of DNA obtained by Laser Capture Microdissection does not allow further molecular biology studies.

Minor Essential Revisions: Few spelling errors (Page 6, row 7 on bracket is missing) (Page 7 row 15 “foci” instead of loci…) but in the Reference list for Journal names should use the recommended
abbreviations (some have the full name and some the Cited form- ref. 1, 13 Journal of Clinical Endocrinology and Metabolism but in ref.14 J Clin Endocrinol Metab).
Also, through the manuscript the gene names should be Italic style.
Figure legend: “Figure 3: DNA sequence analysis of MEN 2 MTC sample with somatic mutation in the VHL gene” but correctly: Somatic mutation of the VHL gene in MEN2A-associated MTC. “on PCR products as described by Ganguly et al” but correctly -on PCR products obtained using PCR conditions described by Ganguly et al.
The quality of Figure 2 is poor.
Page 18, Table 1. in title should include mutations of RET.

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

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

Quality of written English: Acceptable

Statistical review: No

Declaration of competing interests:
I declare that I have no competing interests.