Reviewer's report

Title: Somatic mitochondrial DNA Alterations In Esophageal Cancer: Significance of Novel Missense and Frameshift Mutations and Alteration in mtDNA Content

Version: 1 Date: 7 February 2006

Reviewer: Angela Tan

Reviewer's report:

General
A very good manuscript that contributes valuable information in regards to the role and types of mtDNA mutation in esophageal cancer. Screening the entire mt region is essential and can be made possible using methods such as TTGE. It would be interesting to see the result of larger samples sizes.

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

1. The use of surrounding matched “normal” tissue with tumour samples is not always ideal due to “field” effects. Tissue that appears normal histologically in this vicinity may not be normal on a molecular level. This may explain inconsistencies across the tumours with regards to mtDNA copy number. This may also explain the aberrant A9182G “back change”. This issue will be difficult to resolve but the authors need to indicate that tissues adjacent to the tumour is not always the ideal control tissue for molecular analysis, peripheral blood maybe a better choice. If the normal tissue was obtained from a location the authors feel were sufficiently “normal” this needs to be described in detail.

2. TTGE is a far superior method in regards to detection sensitivity compared to direct sequencing. The authors do not mention how the results from the TTGE results were correlated with sequencing and whether any TTGE results were seen but could not be detected by sequencing.

3. Page 8 in the text description of Figure 1, it is described as showing homoplasmy to heteroplasmy, but this is not true for all of Figure 1 there is a heteroplasmy to homoplasmy change (D).

4. Page 9 in the section “Three had quantitatively different proportion of the mutant…Heteroplasmic mutations have been found in other tumours” It is unclear what the authors are trying to convey. Please clarify.

5. Page 10 “Other more frequent germ-line polymorphisms are…” It is not clear why these particular polymorphisms are mentioned. Perhaps elaborate for clarity or remove from the manuscript.

6. Numbering appears to be inconsistent in regards to the silent novel germline mutations. In Table 2, they are T12957C/G12561A/T7711C. But in the text there is reference to “G9377A in COIII”. Make numbering consistent.

7. Page 11 “All five novel missense…” the authors need to clarify that these were studies that performed previously in separate studies.

8. Page 12 “The PATIENT had a previous history of tongue cancer” Clarify that this is patient E18
9. Page 13 The authors suddenly refer to screening for the presence of large deletions. This should be mentioned in the methods section if it is included in the discussion section.

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Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

1. Some grammatical errors need to be addressed
Page 1 “Division”, not “Devision”
Make sure the font size and type is consistent
Page 5 Somatic mtDNA mutations in THE non-coding
These studies WERE limited
Results from others… cancers SUGGEST that the
…in THE coding region
…content in TUMOURS may have
…age of the PATIENTS…WERE from 38 to 73
Page 7 check font size of website for mitomap
…and those THAT APPEAR in the database
Page 8 THE TTGE mutation detection
THE D-loop involved AN insertion
…THE nt303-309 not np?
Direct sequencing of the PCR fragment rather than DNA fragment?
Page 9 HeTeroplasmic
Page 10 …serine in THE tumour… …change THE amino acid
Page 13 …THE non-coding D-loop
Page 15 …likely to have EFFECTS… or …likely to have AN effect…
…appeared to have EFFECTS in modifying cancer risk or ARE directly INVOLVED in the...

2. Figures 2 and 3 need to be improved in quality. The legends for these figures are not descriptive enough in regards to the colours etc...

3. It might be more convincing if the reverse strand of the sequencing results were also shown in Figure 1 C and D. The deletion position is not immediately apparent to the reader in C. The sequencing result for D is slightly dubious.

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Discretionary Revisions (which the author can choose to ignore)

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: Needs some language corrections before being published

Statistical review: No

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