December 26, 2013

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 6588-review.doc).

Title: Somatic alterations in mitochondrial DNA and mitochondrial dysfunction in gastric cancer progression

Author: Hsin-Chen Lee, Kuo-Hung Huang, Tien-Shun Yeh, Chin-Wen Chi

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 6588

The manuscript has been improved according to the suggestions of reviewers:
1 Format has been updated

2 Revision has been made according to the suggestions of the reviewer

(1) Reviewer 1: *In this review, authors summarize recent findings of the somatic mtDNA alterations identified in gastric cancers, and their relationships with the clinicopathological features of this cancer. They suggest that point mutation and a decrease in copy number of mtDNA are the two most common mtDNA alterations and might result in mitochondrial dysfunction in gastric cancers. The search for strategies to prevent the mtDNA alterations and to block the mitochondrial retrograde signalingwill benefit the development of novel treatments for this and other malignancies. this review is rational and good.*
Ans: We thank the reviewer's comments.

(2) Reviewer 2: *The authors should summarize the views or results of the references, rather than superimposing the original literature content. They should make the review more clear to the readers. They should cite the latest related articles as much as possible.*
Ans: We thank the reviewer's suggestions. We have made revisions and cited several latest related articles as much as possible.

(3) Reviewer 3: *This review is generally well-written and covers an emerging and somewhat controversial topic of the role of somatic mtDNA mutations in cancer in general, and in gastric cancer in particular. However, it can benefit from some language editing and from being more inclusive of alternative viewpoints. Specific comments: (1) the statement that "50% of the identified somatic point mutations in the protein-coding region and the tRNA genes of mtDNA of gastric cancer are potentially harmful mutations" suggests a global conclusion about all mtDNA mutations in gastric cancer. Since this conclusion is based on a single study in which a limited number of mutations was analyzed, this reviewer would like to suggest conducting a similar analysis of mtDNA mutations reported in other studies and to report the outcome in a table format. (2) While earlier studies indeed reported that "the steady-state levels of oxidative damage to mtDNA are several-fold higher than that to nuclear DNA [54-56]", more recent studies (see e.g.. Free Radic Biol Med (1999): 27, 456–462; FASEB J (2000):14,355–360; Ann NY Acad Sci (2005): 1042, 210–220) indicate that steady-state levels of oxidative damage in nuclear and mitochondrial DNA are similar. This needs to be acknowledged to avoid bias. (3) The authors should consider incorporating into review not only arguments for the possible increased oxidative damage to*

mtDNA, but also challenges to the validity of these arguments (see e.g. FEBS J. (2009): 5768-87). Specifically, it has been pointed out that oxidative damage is repaired predominantly by the Base Excision Repair pathway, in which mitochondria are proficient. Moreover, there is evidence that the oxidative lesion 8-oxoguanine is repaired more efficiently in mtDNA than in nDNA (see previous reference). (4) It may be beneficial to clarify how a general defect in DNA polymerase gamma leads to localized mutations in the D-loop and not elsewhere in the mitochondrial genome. (5) It may benefit readers to acknowledge that oxidative damage can result in >20 different DNA lesions, some of which can result in transition mutations.

Ans: We thank the reviewer's comments and suggestions. According to the reviewer's suggestions, the manuscript has been edited for proper English language, grammar, punctuation, and spelling by two of the highly qualified native English speaking editors at American Journal Experts. We also add some alternative viewpoints. For specific comment (1), we have added some references and revised the related sections. For specific comment (2), we have deleted the sentences and rewritten the related paragraphs. For specific comment (3), we have deleted some sentences and rewritten the related paragraphs. For specific comment (4), we have rewritten the related sentences. For specific comment (5), we have added the information and rewritten the paragraph.

For specific comments (2) to (5), the related sections were rewritten as "Given that the mitochondrial electron transport chain is a major site for intracellular ROS formation, oxidative mtDNA damage is predicted to be an important factor promoting mtDNA mutations and genome instability in cancers. However, whether steady-state levels of oxidative mtDNA damage are increased in gastric cancer compared with corresponding noncancerous stomach tissue remains unknown.

The main pyrimidine and purine product of oxidative DNA base damage is thymine glycol and 7,8-dihydro-8-oxo-2'-deoxyguanosine (8-oxodG), respectively[57-60]. Thymine glycol is poorly mutagenic, but 8-oxodG can result in G-to-T transversion mutations during replication because unrepaired 8-oxodG can pair with adenine[61]. However, the most common mtDNA mutations in cancer are transition mutations rather than the mutational consequences specific to 8-oxodG (G-to-T transversion). Therefore, DNA lesions other than 8-oxodG could be primarily responsible for mtDNA transition mutations in cancer. Some studies indicated that oxidative lesion 8-oxodG can be efficiently repaired in mtDNA[62]. In addition, oxidative DNA damage can produce a range of base lesions, and the mutagenic potential of these lesions has not been fully elucidated[63]. In fact, some of these lesions may be responsible for ROS-mediated mtDNA mutagenesis. Moreover, reactive nitrogen species (RNS) can deaminate adenine to hypoxanthine, cytosine to uracil, and guanine to xanthine, thereby causing transition mutations[64, 65]. Thus, it is possible that mtDNA transition mutations in cancer could result from the deamination of adenine, cytosine, or guanine by RNS. Alternatively, factors other than oxidative damage are primarily responsible for the formation of mtDNA mutations, such as defects in mtDNA polymerase or repair systems[62, 66].

Oxidative damage could also contribute to mononucleotide or dinucleotide repeat instability in mtDNA[64]. The mononucleotide repeat in the D310 poly-C sequence of the D-loop region, the most common site of somatic mtDNA mutations in cancer, is the site most susceptible oxidative damage in mtDNA[67]. Moreover, extensive oxidative damage to the mononucleotide repeats may result in slippage and/or misincorporation of nucleotides during mtDNA replication or repair by mtDNA polymerase γ (POLG). Importantly, it has been reported that POLG is a target of oxidative damage[68] and frequently harbors mutations in cancerous tissues[69]. Specifically, mutations were identified in all three domains of the POLG protein, including the exonuclease domain, the linker region and the polymerase domain[64]. In addition, increased mtDNA mutations are observed in *Polg*$^{exo-/-}$ and *Polg*$^{exo+/-}$ mice[70, 71]. Therefore, defects in the polymerase and repair activities of POLG might enhance the generation of mtDNA mutations and genome instability in cancer.

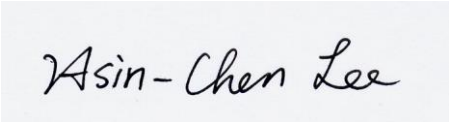
However, whether a general defect in POLG per se leads to increased mutations or genome instability in the D-loop region compared with other region in the mitochondrial genome and the mechanisms governing this action remains unknown."

3 References and typesetting were corrected

4 The manuscript has been edited for proper English language, grammar, punctuation, spelling, and overall style by two of the highly qualified native English speaking editors at American Journal Experts.

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,



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