NKX6.3 protects against gastric mucosal atrophy by downregulating β-amyloid production
Yoon JH et al. This is an interesting paper from experienced investigators postulating that NKX6.3 might play a critical role in the development of gastric mucosal atrophy by regulating Aβ production, and thus that Aβ can lead to gastric atrophy. The concept is novel, the studies were well designed and performed. Several issues, however, need to be clarified and elaborated upon.
Comments:
1. The authors should elaborate more on amyloid peptides. Previous studies (see ref. 1 below) showed that in insulin producing islet cells of the pancreas the toxic activity is mediated by the fibrillar form of the peptide and that neurotoxicity is mediated by the βA fibrils, suggesting that a common mechanism of cell death may operate in many diseases associated with amyloid fibril formation.
Answer: Thank you for your suggestion. As describe in "Results" and "Discussion" section, we analyzed expression of Aβ monomer or oligomers, but not Aβ fibrils. Although the antibody that we used binds to both Aβ peptide and Aβ fibril, Aβ fibrillar form was not detected in western blot analysis using cell line and gastric mucosal tissues. As the reviewer suggested, we elaborate on amyloid peptide in main text.

2. They should provide more detailed information regarding βA. In Figure 3G description they said: "Immunofluorescence analysis showing expression of Aβ oligomer and Bace1 only in gastric mucosae with (W/) atrophy, but not in gastric mucosa without (W/O) atrophy." This is important Figure and should be of better quality and higher magnification allowing to determine whether expression of Aβ oligomer and/or fibrils is localized? - to cells epithelial cells only or also present in extracellular matrix. Congo red staining followed by illumination with polarized light would be very helpful. Do inflammatory cells produce βA?
Answer: 1) As the reviewer suggested, we have improved the resolution and provided higher magnification of the Figure 3G.
2) In immunofluorescence analysis, Aβ oligomer was present only in gastric mucosal epithelial cells, but not in extracellular matrix including inflammatory cells. We have added this sentence in "Results" sections.
3) In general, congo red stain appears to be of limited sensitivity, so that small and tiny deposits of amyloid can be easily missed (Rocken C, Sletten K. Amyloid in surgical pathology. Virchows Arch 2003;443:3–16.) In addition, false positive and false negative results are also related to the fixation. Because Aβ oligomer was detected in the cytoplasm of the gastric mucosal epithelial cells, but not in extracellular matrix, we did not perform congo red staining.

3. Some studies (see ref. 2 below) showed that partial atrophy of the gastric mucosa in aging is not related to the inflammation. This should be discussed in the revised discussion.
Answer: Thank you for your important suggestion. Tarnawski et al has reported that partial atrophy of gastric glands of the gastric mucosa in aging rat is not related to the inflammation. They also found that gastric mucosa of aging rats has increased susceptibility to injury vs that of young rats. In this study, we examined the role of NKX6.3 in gastric mucosal atrophy and found that 1) treatment with rAβ 1-42 produced oligomeric forms of Aβ only in HFE-145$^{shNKX6.3}$ cells, but not in HFE-145^{shCtrl} cells (Figure 4A), 2) treatment with rAβ 1-42 significantly increased floating cell population in HFE-145$^{shNKX6.3}$ cells (Figure 4B

and C), 3) NKX6.3 depletion in HFE-145$^{shNKX6.3}$ cells dramatically increased the expression of inflammatory cytokines and COX-2 (Figure 5A). These results suggest that NKX6.3 depletion might play an important role in Aβ oligomerization and gastric mucosal inflammation, which subsequently contribute to gastric mucosal atrophy. Because a variety of damaging agent results in increased susceptibility of gastric mucosa to injury, it is likely that depletion of NKX6.3, which plays an important role in maintaining gastric mucosal integrity, may account for increased susceptibility of gastric epithelial cells to Aβ-induced cytotoxicity and contribute to gastric mucosal atrophy. We have added the reference in the "Discussion" section.

4. The authors should provide a diagram representing their concept of gastric atrophy and the role of β-amyloid in this process.
Answer: As the reviewer suggested, we have added a diagram representing gastric atrophy and the role of Aβ and NKX6.3 (Figure 6).

This manuscript reports on the role of NKX6.3 transcription factor in the protection of gastric mucosa epithelial cells from atrophy by inhibiting AB peptide production and polymerization. By applying several advanced methods of analysis, the authors demonstrated Ab accumulation in the cytoplasm of HFE-145 cells and gastric mucosa with atrophy, and that NKX6.3 is a key regulator of gastric mucosal homeostasis by inhibiting the cell proliferation and apoptosis. Moreover, based on the obtained results the conclusion is that NKX6.3 suppresses gastric mucosal inflammation by modulation ApoE-induced NFkB and the expression of inflammatory IL-6 and IL-8 cytokines, and COX-2.
Please correct English in the last sentence in the Summary.
Answer: As the reviewer suggested, we have corrected the last sentence in the Summary.