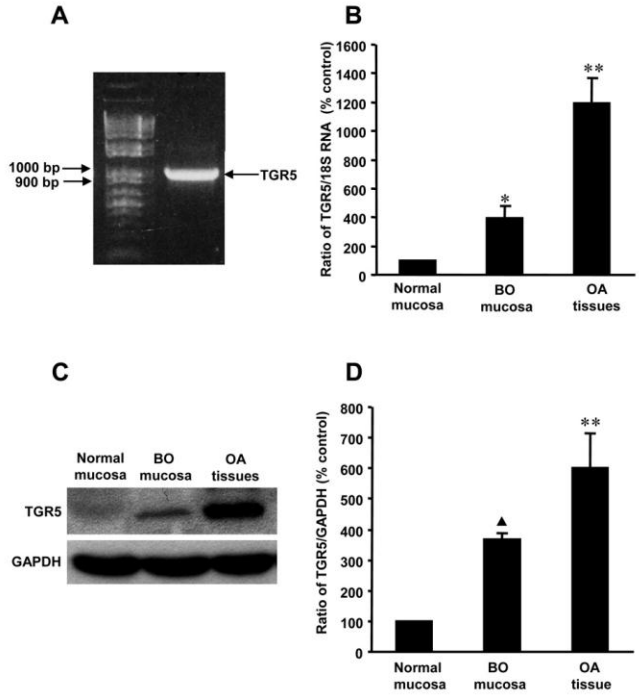
1. I suggest the IHC results be substantiated by qPCR after extraction of RNA from the specimen.

We have measured the mRNA and protein levels in squamous mucosa, Barrett's mucosa and esophageal adenocarcinoma tissues by qPCR and western blot analysis. We found that the levels of TGR5 mRNA and protein expression were significantly increased in Barrett's mucosa (BO), when compared with normal esophageal mucosa. TGR5 mRNA and protein levels are significantly higher in esophageal adenocarcinoma (OA) tissue than in normal oesophageal mucosa or in Barrett's mucosa. The data were published in Gut. 2010;59(2):170-80. For your convenience, we included the figure here:



Because the dysplasia is usually focal, we do not have enough fresh tissues to measure TGR5 mRNA levels in dysplasia. Instead, we measured TGR5 mRNA levels in a dysplastic cell line CP-D. Data are included in figure 1.

2. The results show that the expression of the TGR5 is associated with development of adenocarcinoma and unlike involved in its progression (as same across different stages). This needs to be highlighted in Abstract and discussion. If supported by subsequent studies will be an interesting finding from the current study.

We have added the sentence "TGR5 may play an important role in the progression from BE to EA" in the abstract and the discussion.

3. The sentence ..... "Barrett's esophagus was made by both histologically and endoscopically."..... in section 2.1 seems incomplete

We have modified the sentence and made it clearer.

4. A para needs to be added in introduction to demonstrate role of the RGT5 in carcinogenesis citing latest findings.

We have added the recent findings of TGR5 in the introduction.

6. Discussion needs a serious redrafting. It seems reads reaches again to introduction or results section. No needs to repeat the stuff.

Thank you for your suggestion. We have made some changes according to your suggestion.

The findings - a) differential expression across different developmental grades-b) no significant changes across grades and stages, need a biological explanation.

We have added a sentence in the discussion" These data suggest that TGR5 may not be a marker for the prognosis of esophageal adenocarcinoma."

How the increased expression of RGT5 help a cell to move from normal to a malignant stage?. This needs to be discussed in context of available literature.

We have discussed it on page 9 and 10. "How TGR5 is involved in this progression is not clear. Recently we found that TGR5 mediates bile acid-induced activation of cyclic AMP response element binding protein (CREB) and NADPH oxidase NOX5-S, which produces reactive oxygen species and causes DNA damage. TGR5 is present in human gastric cancers and promotes epithelial-mesenchymal transition in gastric cancer cell lines. It also mediates bile acid-induced cholangiocyte proliferation in vivo and in vitro. Therefore, we speculate that in Barrett's patients bile acids may activate TGR5 receptors, which activate CREB and NOX5-S. NOX5-S-derived ROS may increase cell proliferation and cause DNA damage, thereby contributing to the progression from BE to EA."

What we can infer from no changes in expression of the gene/protein across different grades/stages needs to be discussed.

We have added a sentence in the discussion" These data suggest that TGR5 may not be a marker for the prognosis of esophageal adenocarcinoma."

Do we have evidence to suggest TGR5 a marker of development rather a markers progression in Adenocarcinoma of esophagus, needs to be discussed in the discussion.

We have added a sentence in the discussion on page 10 "TGR5 might be a potential marker for the progression from BE to high-grade dysplasia and EA."