Reviewer’s report

Title: Long non-coding RNA, LEIGC, plays as tumor suppressor in gastric carcinoma through inhibiting epithelial-to-mesenchymal transition

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Reviewer: Joana Carvalho

Reviewer’s report:

This manuscript by Han et al, pinpointed a novel long-noncoding RNA, LEIGC, as a potential tumour suppressor in gastric cancer. By using molecular and functional assays, the authors found that LEIGC was downregulated in gastric tumours in comparison with adjacent non-tumour tissue and regulates the growth, proliferation, migration, EMT and sensitivity to 5-Fu of gastric cancer cells.

Long non-coding RNAs have been implicated in gastric cancer, nevertheless the expression and function of LEIGC was never found and functionally explored in gastric cancer. From that point of view, alone, this manuscript is interesting. However, after having carefully reviewed the manuscript and figures, I feel that several minor points should be clarified.

The authors should considering the following:

Minor essential revisions

1- Abstract
The authors should better clarify the aim of the study. In addition, some sentences should be more carefully written.

2- Background
In general, the introduction is organized although some sentences should be more carefully written and references should be added particularly in sentences 79-80; 81-82. In addition, some studies describing the role of long non-coding RNAs in gastric cancer should be mentioned with more detail.

3-Materials and Methods

“LncRNA expression microarray analysis” - the authors should clarify if the LncRNA microarray includes probes for non-annotated lncRNAs and which lncRNA database was used in the genesis of the array.

“Quantitative real time PCR analysis” – the authors should describe the method used in the analysis. In addition, instead of protein names, gene names should be adopted (e.g CDH1 instead of E-cadherin.

“Western blotting” – Please mention the clone used for each antibody and the respective dilution.
4- Results
Expression of LEIGC in gastric cancer tissues – The authors should mention how many up and downregulated lncRNAs were found to be differentially expressed in gastric cancer tissues compared to non-cancer tissue. It would be nice to see a table containing the differentially expressed lncRNAs accompanied by the fold change and p value.

Figure 1 should be more carefully presented. Please consider the following:
1) An heatmap scale and a dendogram for samples should be presented on figure 1A;
2) The legends of y-axis of figures 1B, 1C, 1D should be written
3) The data presented on figure 1D was not described in the results section. Is there any mistake?
4) It would be nice to see the expression levels of LEIGC in T and N presented as Relative Quantity (RQ) of #CT. RQ=2^(-#Ct) ; #Ct= Ct LEIGC- Ct GAPDH.

Structure analysis of LEIGC
The authors mentioned that LEIGC has two exons. It was not clear how did the authors found these two exons. Which software was used to state that LEIGC has two exons? In the UCSC gene browser, LEIGC overlaps with a clone ID: AC009312.4. Is there any relationship between LEIGC and this clone?

LEIGC inhibits migration of gastric cancer cell in vitro
It would be nice to see if LEIGC also regulates the invasive capacity of gastric cancer cells by performing a Matrigel transwell invasion assay.

LEIGC enhances the chemosensitivity to 5-Fu in gastric cancer
In the last part of this section, the authors measured the IC50 values to 5-Fu in three different gastric cancer cell lines transfected with shRNA for LEIGC. Since the authors have already created these models, it would be nice to see the impact of LEIGC knockdown on the growth, proliferation, migration and invasion capacity of SGC-7901 and AGS cells.

LEIGC, a novel factor that prevents EMT in gastric cancer
In this section the authors demonstrated that loss of LEIGC expression was accompanied by a transition from an epithelial to mesenchymal morphology as well as gain and loss of well-established mesenchymal and epithelial markers, respectively. It would be nice to quantify the mRNA expression levels of the same mesenchymal and epithelial markers by doing quantitative real-time PCR in the same cohort of gastric tumours and determine if there is any correlation with the expression levels of LEIGC.

Did the authors have any hypothesis for the role of LEIGC in EMT? Did LEIGC regulate direct or indirectly E-cadherin or other EMT associated proteins? RNA pull-down and immunoprecipitation assays may help on this issue.
5- Discussion

In general the discussion should be more organized and the authors should discuss their results based on recently published data on the expression and function of long non-coding RNAs in gastric cancer. In addition, it would be nice if the authors advance some hypothesis concerning the mechanisms underlying LEIGC downregulation in gastric cancer as well as its mode of action.

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, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

I declare that I have no competing interests