Nov 1, 2012

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: +Revised_Paper_JCOTang_World_J_Gastroenterology.doc).

Title: Oncogene GAEC1 Regulates CAPN10 Expression which Predicts Survival in Esophageal Squamous Cell Carcinoma

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Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 394

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

Revision has been made according to the suggestions of the reviewer

(1) "The authors should find a correlation with another markers, or at least describe other markers, at the histological level."

In order to assess whether the tumors are more proliferative, the use of other histological markers for assessing proliferation, such as Ki-67(Brown and Gatter 2002) and AgNOR(Derenzini 2000), in parallel to CAPN10 is suggested in future studies to determine whether the CAPN10 level is associated with progression of the disease.

This point has been added to the discussion part of the amended paper.

(2) "describe not only the staining but also describe the tumor at histological level."

The histology of tumors of figure 4 has been described.

(3) "maybe information about the amplification of the chromosomal segment 7q22 could be studied."

From our previous study(Law et al 2007), no significant correlation was observed between GAEC1 amplification and clinicopathological parameters and prognosis in ESCC tumors, and thus the DNA amplification of GAEC1 is not included in the present study.

This point has been added in the revised manuscript.

(4) "Staining data with TNRC6C should be shown, with the TMA, but also with positive controls for the staining, to exclude the possibility that this negative data could be due to technical problems."

The description about the comparison of the TNRC6C-stained cases with the positive control (eight non-tumor esophageal tissues) was added in the Results section.

(5) "to address the expression of CAPN10 in metatstasis."

The level of CAPN10 is not associated with local lymph node and distant metastasis in the ESCC cases, implying the possibility that GAEC1 expression is not relevant to the control of metastasis in ESCC.

This point has been added to the discussion part of the amended paper.

(6) "survival analysis in patients of ESCC should be addressed more in the methods and results section"

The survival range (0.72 months to 65.15 months) of the studied patients was added to the result section. The survival analysis has been described in methodology section under "Statistical Analysis".

(7) "The authors might state their reasons about the findings of having no significant correlation of any of the clinicopathological features with the expression level of CAPN10, but conclude the low expression of CAPN10 predicted the poor survival of ESCC patients., providing a reference, if available."

Similar results were reported previously in which the overexpression of a chemokine CXCL12 in ovarian cancer(Popple et al 2012) and a protein Rad51 for homologous recombination in ESCC(Li et al 2011) also showed a correlation to the survival of patients, but no correlation to other clinicopathological features was found.

This part has been added to the discussion section.

(8) "add some data about the protein expression levels of GAEC1 in KYSE150 cells by Western blot or immunofluorescence assay."

This point is suggested in the discussion section. This work is feasible if there is GAEC1 antibody available in the market.

(9) "Ethical approval should be mentioned"

The use of archival paraffin-embedded ESCC tissues was used under the ethical guidelines in the Department of Pathology of The University of Hong Kong.

This part has been added to the methodology section.

(10) "Several spelling mistakes (e.g. on page 10, "5 to 25<%" "25 to 50<%"should correct for "5% to 25<%" "25% to 50 %< %"; on page 13 "p=0.032" should correct for "P=0.032")."

The text has been amended as suggested

(11) "provide the data of "survival analysis"."

Data of survival analysis is provided as follow, but it is too lengthy to be shown in the text of the paper:

Overall Comparisons

	Chi-Square	df	Sig.
Log Rank (Mantel-Cox)	4.625	1	.032
Breslow (Generalized Wilcoxon)	3.891	1	.049
Tarone-Ware	4.385	1	.036

Test of equality of survival distributions for the different levels of CAPN10.

(12) "Figures 4 should be added images of immunohistochemical staining of TNRC6C and combined as new Figures."

The representative images of TNRC6C staining have been added to Figure 4

(13) "Please provide "percent" values in Tables 3 as well as in the results describing these data."

Percent values have been added in Table 3 and inside text

(14) "What new information does this work provide relative to Moreno-Luna R et al, 2011(Ref.32)? The authors should discuss this point ("CAPN10 expression predicts survival in ESCC") in more detail."

From the study of Moreno-Luna R et al.(Moreno-Luna et al 2011), CAPN10 genotype 12 was reported to be related with a worse prognosis in laryngeal cancer, which is similar to our present study and this is newly described in ESCC.

This part has been added to the discussion section.

(15) "on page 15, 16, "(Fig. 4)" and "(Table 1)" should be deleted."
Deleted

(16) "The authors should check the entire manuscript for spelling errors"

Spelling errors were checked.

(17) "Reference needs to be further updated."

5 references have been updated.

(18) "Do the authors consider the result from only cell line enough to conclude the function of GAEC1?"

Our group also investigated several ESCC cell lines showing overexpression of GAEC1, but only KYSE150 showed the more stable and consistent overexpression with time. Thus for this first study of GAEC1 suppression, only KESE150 was used.

This part has been added to the discussion section.

(19) "Did the authors take the intensity into consideration in the immunohistochemical staining for calpain 10 and TNRC6C?"

Yes, this point has been described in the methodology section.

(20) "Figure 1 seems to reveal the suppression but it is not quantitative analysis scientifically."

Densitometry analysis was done on the intensity of GAEC1 bands from the photo using Quantity-One program (Bio-Rad) and the reduction in band intensity of GAEC1 was confirmed.

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

Sincerely yours,

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