Peer Review File

Article Information: http://dx.doi.org/10.21037/jgo-20-617

Comment 1 by Reviewer A

Fig.4 may be revised to indicate the differences of KRAS expression between intestinal gastric adenocarcinoma and signet ring cell carcinoma. It is not clear why KRAS expression decreases in IHC- and +, and increases in IHC ++ and +++ in signet ring cell carcinoma in Fig4B. Y axis may be changed. Careful proofreading and revision in conclusion may be needed.

Reply 1： Fig.4 indicated the difference of IHC staining of KRAS expression levels in our two internal cohort. Firstly, we put negative (-) or weak positive (+) expression into a lower expression group, median (++) or strong positive (+++) expression into another higher expression group. Secondly, we compared the expression difference between the two group. In SRCC cohort, 54 patients were detected with negative (-) or weak positive (+) expression, whereas 21 patients were KRAS median (++) or strong positive (+++). In intestinal cohort, 50 patients were detected with negative (-) or weak positive (+) expression, 7 patients were KRAS median (++) or strong positive (+++). Obviously, the majority of SRCC patients were median or strong positive KRAS expression, which is higher than our intestinal cohort (28% vs 12.6%, P = 0.033, Fig 4). （see Fig.4B）

Changes in the text: We have modified Fig.4B to make it easier for readers to understand.

Comment 2 by Reviewer B

Abstract: lines 29-30, the various abbreviations like TCGA, IHC, FISH and FFPE must be explained in full name.

Reply 2: We have modified and added the explanations of the abbreviations in full name. （see Page 2,line58-60）

Changes in the text: The Cancer Genome Atlas(TCGA), Immunohistochemistry(IHC) , Fluorescence in situ hybridization(FISH), Formalin-Fixed and Parrffin-Embedded (FFPE).

Comment 3 by Reviewer B

Lines 43- 44, the authors did not mention that these cell lines are of SRCC origin and also must put the names of 2 other cell lines that they used

Reply 3: We used four gastric SRCC cancer cell lines (SNU601, SNU668, KATO-III and NUGC-4). KATO-III cell lines were obtained from the Chinese Collection of Research Bioresources. Other 3 cell lines (SNU601, SNU668, and NUGC-4) were provided by the National Cancer Center Research Institute (Tokyo, Japan) in 2011. We have modified and added the names of 2 other cell lines we used. (see Page 3,line79）

Changes in the text: SNU601 and SNU668, which harbored KRAS mutation, were hypersensitive to MEK and mTOR inhibitors than KRAS wide type cell lines KATO-III and NUGC-4.

Comment 4 by Reviewer B

Material and methods : line 112, the number of samples may be mentioned.

Reply 4: We have modified and added the number of samples in the text. (see Page 7,line 138-147）

Changes in the text: We added in the part of Patients and samples: This study included 234 SRCC patients and 57 intestinal gastric adenocarcinoma patients who underwent gastrectomy at the General Surgery Department of Drum Tower Hospital between 2010 and 2016.

We added in the part of Immunohistochemistry:75 SRCC patients and 57 intestinal gastric adenocarcinoma patients in our internal cohort which had sufficient tumor tissues were fixed in formalin and embedded in paraffin.

Comment 5 by Reviewer B

lines 123-127, the scale between 0-3 and their% is not clear.

Reply 5: We have modified the literal expression to make the standard more clearly. (see Page 8,line160-164）

Changes in the text: KRAS expression was evaluated semi-quantitatively according to the degree and the proportion of membrane staining at the same time . 0 manifested no staining is observed or membrane staining is observed in <10% of tumor cells; 1+ mean faint or partly membrane staining is found in >10% of tumor cells; 2+

represented weak to moderate complete membrane staining is detected in >10% of tumor cells; 3+ represented strong, complete membrane staining is observed in >10% of tumor cells.

Comment 6 by Reviewer B

line 162, the composition of the culture medium needs to be more precise. Line 165, the drugs properties must noted.

Reply 6: We have modified and added the detailed culture medium. (see Page 11,line208-216）

Changes in the text: Cells were seeded in 96-well plates (3000 cells per well) with antibiotic-free RPMI 1640 (Invitrogen) plus 10 % fetal bovine serum at 37 °C with 5 % CO2 for 24 h. The cells were then treated with AZD6244 and AZD2014 for another 72 h to determine the 50 % inhibition concentrations (IC50). The growth-inhibitory effects of AZD6244 and AZD2014 were tested by 3, 4,5-dimethyl-2H-tetrazolium bromide assay (MTT; Sigma-Aldrich). Optical density was spectrophotometrically measured at 570 nM. Each experiment was carried out in triplicate and data are presented as geometric means

Comment 7 by Reviewer B

Results: lines 192-193, the TCGA samples have very few patients with SRCC. and authors need more explanation on the methodology of their choice.

Reply 7: We did data analysis by using TCGA STAD datasets. Both mutation data and clinical information were looked up. There are 8 histological types, including Adenocarcinoma with mixed subtypes, Adenocarcinoma of intestinal type, Adenocarcinoma NOS, Carcinoma of diffuse type, Mucinous adenocarcinoma, Papillary adenocarcinoma, Signet ring cell carcinoma, Tubular adenocarcinoma. Among 388 patients with mutation data, only 11 patients were diagnosed with Signet ring cell carcinoma. We have modified and added the detailed methodology of choice. (see Page 13,line245-246）

Changes in the text: There are 8 specific histological types in the TCGA STAD dataset. Among the 388 patients, only 11 patients were pathologically diagnosed as SRCC.

Comment 8 by Reviewer B

Line 206 and table 3. Contrary to ideas received in the literature which is also noted in the introduction to this article, SRCC tends to be more common in women than in men; but here the authors find present different results. of 234 SRCCs, 60 are women. and this tendency may added in discussion

Reply 8: We have added the analysis and probable reason in discussion. (see Page 16,line307-310）

Changes in the text: Previous studies have shown that SRCC tend to occur more often in younger women groups. However, 60/234(25.4%) patients were women in our cohort. Our cohort was selected from gastric cancer surgery samples. Due to the advanced clinical stage, the majority of patients might not receive surgical treatment, resulting in a gender bias.

Comment 9 by Reviewer B

the discussion is well done but authors can assemble the work regarding KRAS G13D Mutation and Sensitivity to Cetuximab or Panitumumab in a Colorectal Cancer Cell Line Model.

Reply 9: We have added the associated work and reference in discussion. (see Page 17,line320）

Changes in the text: Mutations in codons 12 and 13 are correlated with poor overall survival for colorectal cancer patients and are predictors of resistance or sensitivity to EGFR-targeted therapy by activated both the PI3K/AKT and MAPK signaling