March 4, 2013

Dear Su-Xin Gou, Science Editor

Please find enclosed the edited manuscript in Word format (file name: wjg-2012-000136-corrected.doc).

Title Underexpression of *LATS1* TSG in colorectal cancer is associated with promoter hypermethylation

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Name of Journal: *World Journal of Gastroenterology*

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

1) P values were corrected, showing relevant values if needed.
2) The graphs are corrected and decomposable.
3) Since language certificate letter was suggested, the BioMed reviewer brought in some substantial corrections, where the most important are:
    a. Change the orientation of Table 1.
    b. BioMed suggested to change the manuscript title, however it could not be done.
4) Revision has been made according to the suggestions of the reviewer
    a. We corrected and shortened the abstract as suggested, showing only Materials and methods and Results. However, the BioMed reviewer strongly suggested to maintain a proper form based on BPG's Revision Policies for Brief Articles. Thus we decided to keep the recommended form (Aim, Methods, Results, Conclusion) but with less words.
    b. MSI study: we analyzed group to 84 CRC cases with obtained venous blood including 57 cases from our previous data on MSI status (manuscript attached, written in polish, however abstract and table descriptions are in English). MSI status was carried according to National Cancer Institute Workshop on Microsatellite Instability for Cancer Detection and Familial Predisposition and was based on polymorphism analysis of 3 markers: BAT26 for MSH2 gene, BAT25 for c-kit oncogene and BAT40 for HSD3B2 suppressor gene. Unfortunately we could not perform IHC study for MLH1, MSH2, MSH6, and PMS2 proteins since we used all tissues for RNA and DNA analyzes. The MSI results are included in the manuscript, expanding the knowledge of MSI status among Polish CRC patients. Also we checked that underexpression of *LATS1* was not associated with MSI status as presented in added Figure # 2 and in Table 2.

c. Methylation status: In order to check the methylation status of CRC, we were supposed to check the occurrence of methylation within CACNAG1, SOCS1, RUNX3, NEUROG1, and MLH1 markers. Unfortunately we failed, but the reasons of failure were partially independent of us. Since we did not have much time we tried two methods to obtain results: bisulphide modification followed by MSPCR and then agarose gel electrophoresis or COBRA technique (MSPCR followed by meth-specific digestion and acrylamide electrophoresis). We did not get any data even after purchasing fresh kits. The age of samples was probably the main reason of failure; we had been collecting CRC tissues and isolating DNA since 2008 to 2011. After bisulphide modification the DNA (modified and unmodified) was stored in -80ºC until requested methylation study. The bisulphide modified DNA was completely physically degradated (such DNA remains intact for not more than 6 months), and the yield and quality of unmodified DNA was too poor to perform bisulphide conversion followed by MSPCR.
d. QPCR: our manuscript was checked by two experienced scientists from our lab who found no methodological or factual errors. Furthermore, it was also corrected by BioMed reviewer.
e. TNM study: we split CRC cases into TNM groups according to AJCC/UICC and MAC divisions. That gave us 8 different groups, however after statistical analyzes we did not find more significant relationships than when we used Dukes' division into 4 groups. Thus we decided to preserve the original division.
f. All statistically significant P values are presented as $P < 0.05$, except for the Table 2 due to BioMed corrections.
g. Discuss: we applied blood results from CRC cases, which is shown in Table 1. We found that RBC results were correlated with tumor's location, however that was not a goal of this study. We patiently have been collecting patients' follow-up, but since the patients were not obligated to contact with us so we have maintained good contact with less than 50 persons and such follow-up study has not yet finished. We included in the manuscript new data regarding the role of LATS1 and SWH pathway in cancer development. The recent results of LAST1 in glioma and new pathway in CRC confirmed that the study on selected gene presented in this manuscript may expand the knowledge of CRC progression.

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

Sincerely yours,

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