Format for ANSWERING REVIEWERS

September 13, 2013

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 4520-review.doc).

Title: Epidermal growth factor uregulates messenger RNA and protein levels of Skp2/Cks1 and p27kip1 in human extrahepatic cholangiocarcinoma cells

Author: Ja-yeon Kim, Hong Joo Kim, Jung Ho Park, Dong Il Park, Yong Kyun Cho, Chong Il Sohn, Woo Kyu Jeon, Byung Ik Kim, Dong Hoon Kim, Seoung Wan Chae, and Jin Hee Sohn MD

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 4520

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

2 Revision has been made according to the suggestions of the reviewer

(1) Is Skp2 alteration specific to extrahepatic cholangiocarcinoma? Does this kind of change happen in extrahepatic cholangitis?

Answer) We enrolled the patients with extrahepatic cholangiocarcinoma and exclude the patients with intrahepatic cholangtiocarcinoma. Hence, we could not check the Skp2 alteration in tissue specimens of patients with intrahepatic cholangiocarcinoma. The previous study for 74 patients with mass forming type intrahepatic cholangiocarcinoma by Hashimoto et al **(**Annals of Surgical Oncology 2009; 16: 395-403) have shown that a statistically significant correlation was found between low p27kip1 expression and lymph node metastasis (P = .009). Patient survival in the low p27kip1 expression group (n = 25) was also significantly worse than that in the high p27kip1 expression group (n = 49, P = .0007). A significant inverse correlation was found between p27kip1 and Skp2 expression (P = .016). High Skp2 expression (n = 36) was significantly associated with poor prognosis (P = .0046). The multivariate analysis revealed that low p27kip1 and high Skp2 expression are independent and significant factors of poor prognosis.However, there were no studies evaluating the expression status of Skp2/Cks1 and p27kip1 and assess the significance of Skp2/Cks1 expression with $p27^{kip1}$ as a predictive prognostic marker in patients with extrahepatic cholangiocarcinoma, so far. The immunoreactivities of Skp2/Cks1 were absent to very weak in the microscopic field of inflammatory cells infiltration site in the relevant microarray tissue blocks.

(2) Does the in vitro study using EGF and cancer cell line really represent the in vivo scenario?

Answer) In vitro study could be supplemented in vivo study and vice versa. First of all, two- dimensional cell culture system is totally different from in vivo three dimensional tissue structure composed of various cell components and extracellular

matrix. Additionally, physiologic dosage of EGF in normal tissue and pathologic dosage of EGF in neoplastic tissue could be different in dosage used in the cell culture system. Hence, in vitro cell culture system used in our experiment may not represent real in vivo system in the human neoplastic tissue. We have a plan to perform further experiments adopting three-dimensional cell culture system more resemble real in vivo environment and animal model to verify the hypothesis proposed in this study.

(3) Some figures and figure labels are hard to see.

Answer) We increased the resolution (dpi) of our figures and figure labels (Please refer to appended powerpoint files).

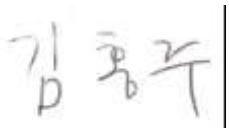
(4) Some typos in the title and text

Answer) We corrected the typos in the title and text using editing function of word program.

3 References and typesetting were corrected

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

Sincerely yours,



Hong Joo Kim, MD, PhD
Dept. of Medicine
Sungkyunkwan University Kangbuk Samsung Hospital
108, Pyung-Dong, Jongro-Ku
Seoul, Korea
Fax: +82-2-2001-8360
E-mail: hongjoo3.kim@samsung.com