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Reviewer A

1. Over-expression studies are good but siRNA knockdown results are not that impressive. Stable knockdown may be more useful

Reply) The authors agree with the reviewer's opinion. Currently, we are producing stable cell lines of ERH using CAS-9. Future studies will use this stable cell line to conduct further sub-mechanism analyses. We have added these comments to the limitations of this study in the discussion section.

In addition, further studies on mechanism of ERH related to prognosis through stable knock-out cell line and in-vivo experiments will be needed. (discussion, page 9)

2. Has the alteration in ERH on tumor growth been tested using xenograft models?

Reply) The authors agree with the reviewer's opinion. In future study, we plan to proceed with the demonstration of additional functions of ERH through by implanting tumors in nude mice. We have added these comments to the limitations of this study in the discussion section.

Regardless, further studies on the mechanism of ERH related to prognosis through a stable knockout cell line and in vivo experiments are needed. (discussion, page 10)

3. Several typos were found in the manuscript that needs to be checked and corrected.

Reply) A professional editing company edited the manuscript for English language.

4. Legends to figures need to be enhanced

Reply) We edited the figure legends.

Figure legends

Figure 1. Results from immunohistochemical analysis of ERH expression in gastric cancer tissues. Staining with an anti-ERH antibody was scored and sorted into groups according to the percentage of ERH-positive cells as follows: (A) 2+ reactivity (25–49%), and (B) 3+ reactivity (50–74%). Arrows show representative cells with positive staining for ERH. Scale bar, 100 μ m. ERH, enhancer of rudimentary homolog.

Figure 2. Underexpressed ERH is associated with a shorter survival time. The ERH overexpression group showed a longer cumulative survival time compared with the

ERH underexpression group according to the results of Kaplan-Meier survival analysis.

Figure 3. ERH overexpression in 2 SNU-601 cell groups (oeERH-1 & 2) and 2 MKN-28 cell groups (oeERH-1 & 2) was confirmed using Western blot analysis (A). Cell proliferation was determined by an MTT assay (B). Wound-closure rates were measured (C). Invasive ability was evaluated using a Transwell assay (D) with overexpressed ERH in SNU-601 and MKN-28 cells and their respective controls. Data are presented as the mean \pm SD for triplicate independent experiments, and the error bars indicate SD.

Figure 4. Depletion of ERH expression in 2 SNU-601 cell groups (siERH-1 & 2) and 2 MKN-28 cell groups (siERH-1 & 2) was confirmed using Western blot analysis (A). MTT assays were used to evaluate proliferation in SNU-601 and MKN-28 cell lines (B). Cell migration was evaluated using a wound-healing assay (C). Invasive ability was evaluated using a Transwell assay (D). Data are presented as the mean \pm SD for triplicate independent experiments, and the error bars indicate SD.

5. References should be checked for accuracy

Reply) We checked the references accordingly introduction for authors.

Reviewer B

1. The most interesting results of invasion and migration are compared only with a study on bladder cancer. Is there only this study in the literature? if so, make clear the absence of studies evaluating this function.

Reply) As recommended, we searched again and found a recent paper on ERH prognosis potential for ovarian cancer, which was added to the discussion section.

In addition, a recent study of ovarian cancer revealed the potential of ERH as a marker for a poor prognosis (33). Inhibition of ERH expression slows proliferation, promotes apoptosis and inhibits metastasis and invasion by regulating epithelial-mesenchymal transition in ovarian cancer cells. (discussion, page 10).

2. Another important point to be discussed is the different behavior of the MKN74 lineage whose migratory and invasive behavior is not altered after oeRNAs treatment. What can explain the discrepant behavior between the strains?

Reply) Your point is quite reasonable.

These result seems to be due to the difference in the characteristics of the two cell lines used in the experiment. MKN74 is a gastric cancer cell line with good differentiation, and SNU601 is a gastric cancer cell line of signet ring cell origin with relatively poor differentiation. Although it is not a significant difference in MKN74, a

slight decrease in invasion can be seen, and knockdown siRNA caused an increasing pattern in both cell lines. I think this can prove the validity of our experimental results.

3. In figure 3, in the scratching assay, only the 48h result is shown. What happened at 6, 12 and 24h? What is the cells doubling time? After 48 the proliferation could confusing the interpretation of the results?

Reply) According to data from Korea Cell Line Bank, the population doubling time of MKN74 is 46-48 hours, with 48 hours showing the greatest difference. The result of the experiment showed 48 hours, which is different from the start time of 0 hours. As you can see from Figure 3B, proliferation was not different from the control, so it is unlikely to confuse the results. I think this should be viewed entirely as a difference in migration.

4. In each assay described in the methodology, the authors must indicate the number of replicates and the number of independent experiments carried out

Reply) Each experiment was independently conducted three or more times. This was added to the experimental method section.

Values for cell invasion are expressed as the mean number of cells per microscopic field over five fields per filter for triplicate experiments. The experiments were performed in triplicate at least three times independently. (materials and method, page 6)

5. Figure 1 needs a scale and the caption should indicate the magnification. I suggest including in the upper right corner a zoom of the histological cut showing, in a better way, the nuclear staining of ERH.

Reply) As recommended, a scale ruler was inserted.