Dear reviewer # 00225313

Thank you very much for your comments and recommendations on our manuscript (World Journal of Gastroenterology; Manuscript NO: 51786) entitled "MicroRNA 375 suppresses MTDH-mediated signaling in colorectal cancer"
With regard to your comments and recommendations, we carefully carried out revised a part of our manuscript.

Revised parts are as follows:

Comment 1. The work presents no major flaws. The authors should stress the limited number of patients and healthy donors enrolled.

Revision: Thank you for your nice comment. We absolutely respect your opinion.
Here is long story for this study. My lab is a small research team consisting of two doctoral students and me. This study was conducted in cooperation with the members of our laboratory and several clinical professors. About 9 years ago, several microRNAs associated with human CRC were identified in my lab by genomic tools, and the results were briefly confirmed using four pairs of CRC samples (Mo et al., 2015). We also briefly validated MIR375 levels using additional four pairs of CRC tissues in previous study (Alam et al. 2017). In the meantime, we conducted the research after receiving IRB review three times. Our University IRB is recommended to use as few samples as possible, because this study is not clinical base study. Place understand this.
According to your comments, we added new sentence, [Sample size was estimated using the G*power software (Version 3.1., Heinrich Heine University, Duesseldorf, Germany).] at Statistical analysis part of Material and Method section.

Dear reviewer # 00058340

Thank you very much for your comments and recommendations on our manuscript (World Journal of Gastroenterology; Manuscript NO: 51786) entitled "MicroRNA 375 suppresses MTDH-mediated signaling in colorectal cancer"

With regard to your comments and recommendations, we carefully carried out revised a part of our manuscript.

Revised parts are as follows:

Comment 1. Title:" MicroRNA 375 suppresses MTDH-mediated signaling in colorectal cancer" Since the MIR375 is significantly downregulated in human CRC it would be better to rephrase this title to: Reduced MicroRNA 375 in colorectal cancer activates MTDH signaling or similar.

Revision: Thank you for your nice comment. According to your comments, we changed the title from [MicroRNA 375 suppresses MTDH-mediated signaling in colorectal cancer] to [Reduced microRNA 375 in colorectal cancer upregulates MTDH-mediated signaling] at Title page.

Comment 2. The authors should elaborate on the mechanisms of MIR375 downregulation in human CRC or at least discuss this in the discussion.

Revision: Thank you for your precise comments. Currently, we cannot explain this mechanism elaborately, but a reference paper is likely to help.

Based on your comments, we added a sentence, [Hyper-methylation of MIR375 has been demonstrated in CRC cell lines including HCT116 and SW480. Down regulation of MIR375 in HCT116 and SW480 cells compere to HT29 cells is due to hyper-methylation of MIR375 in HCT116 and SW480 cells[25]], at Discussion section. We also added a reference and changed the References order from 26 to 40, and shift References in order.

25 **Christensen LL**, Holm A, Rantala J, Kallioniemi O, Rasmussen MH, Ostenfeld MS, Dagnaes-Hansen F, Øster B, Schepeler T, Tobiasen H, Thorsen K, Sieber OM, Gibbs P, Lamy

P, Hansen TF, Jakobsen A, Riising EM, Helin K, Lubinski J, Hagemann-Madsen R, Laurberg S, Ørntoft TF, Andersen CL. Functional screening identifies miRNAs influencing apoptosis and proliferation in colorectal cancer. *PLoS One* . 2014;**9**:e96767.

Comment 3. The authors stated that "MIR375 regulates cell proliferation, cell migration, and angiogenesis by suppressing MTDH expression in CRC progression. MTDH promotes an invasive phenotype and angiogenesis via the PIK3CA-AKT signaling pathway". Please elaborate more on regulation of angiogenesis by MIR375 in the revised discussion. Is angiogenesis increased by inducing VEGF generation by CRC, or by a direct effect in endothelial cells?

Revision: Thank you for your elaborate and invaluable comment. Our previous results using xenograft mouse model showed that the expression level of the angiogenic marker CD31 was significantly decreased by MIR375-mediated PIK3CA-AKT signaling as mentioned in this paper (Alam et al., 2017). The results of previous and present studies led us to check the VEGFA levels by MIR375 mimic and siMTDH transfection in this study (Figure 3). We think that the generated VEGFA by MIR375-mediated PIK3CA-AKT or MTDH-PIK3CA-AKT signaling might effect to endothelial cell's angiogenesis.
According to your comments, we add the sentence, [Although we did not show MIR375-mediated VEGFA-VEGFR signaling in this study, our previous and present studies suggest that the generated VEGFA by MIR375-mediated PIK3CA-AKT or MTDH-PIK3CA-AKT signaling might effect to endothelial cell's angiogenesis.], at Discussion section.

Comment 4. While the paper is well written some minor linguistic corrections should be made, e.g., MTDH was significantly downregulated on siMTDH transfection should be 'by", etc.

Revision: Thank you for your nice comment. Actually, our document was improved by the professional scientific research paper editing Service Company (http://www.editage.co.kr) before submission. But we don't know they are scientific native speaker or not.
Base on your comment, we changed sentence from [Furthermore, MTDH was significantly downregulated on *siMTDH* transfection (Figure 1D).] to [Furthermore, MTDH was significantly downregulated by *siMTDH* transfection (Figure 1D).] at the part of MIR375

regulate MTDH expression in CRC cells of Results section. We also carefully re-checked our document.

Comment 5. Figure 6 titlle: “A simple putative mechanism of MIR375……” should be changed to “ Diagrammatic representations of putative mechanisms of MIR375….

Revision: Thank you for your precise and constructive comment. According to your comments, we changed the title from [A simple putative mechanism of *MIR375* in regulating MTDH-induced cell proliferation, cell migration, and angiogenesis in human CRC] to [Diagrammatic representations of putative mechanisms of *MIR375* in regulating MTDH-induced cell proliferation, cell migration, and angiogenesis in human CRC] at Figure 6.

Dear reviewer # 00068559

Thank you very much for your comments and recommendations on our manuscript (World Journal of Gastroenterology; Manuscript NO: 51786) entitled "MicroRNA 375 suppresses MTDH-mediated signaling in colorectal cancer"
With regard to your comments and recommendations, we carefully carried out revised a part of our manuscript.

Revised parts are as follows:

Comment 1. In this study, the author investigated that MIR375 suppresses MTDH-mediated signaling in colorectal cancer. We have known that MIR375 is suppressed in some solid tumors, such as esophageal adenocarcinoma and gastric cancer. The manuscript is helpful for us understanding how MIR375 regulates MTDH-mediated signaling pathways in CRC progression. In the results, endogenous expression of MIR375 was tested in CRC cell lines, including HT29 cells having the highest expression level. How about MTDH in HT29 cells? It would be better to give a description.

Revision: Thank you for your nice comment. We entirely agree with your opinion. We also wondered about it at the start point of this study. The MTDH expression levels were slightly down-regulated (not significant) in HT29 cells than other CRC lines. Although MIR375 level was higher (about 2~3 folds) in HT29 cells, it is not enough to see a large difference for their target genes. When introduce (transfection) MIR375 into CRC lines, the several ten thousand fold of MIR375 were working (in our previous paper; Alam et al., 2017).
In this study, we did not used HT29 cells as the main cell line. Because HT29 cells have the mutation on BRAF and PIK3CA genes (Ahmed et al., 2013), that are main molecules for this study.
Place understand this.