**Dear scientific editor Ya-Juan Ma and other editors:**

Firstly, we would like to express our great thanks for your positive evaluation for our manuscript titled as "**MicroRNAs: novel immunotherapeutic targets in Colorectal Carcinoma**" (Manuscript NO. 25864). Every author also expresses sincere thanks for giving us a chance to revise our manuscript.

We found the comments were concise and helpful. According to the issues raised by reviewers, we have carefully revised our manuscript and given a point-by-point response as outlined below. We greatly appreciate if you take the consideration of our revised manuscript into publication.

All authors involved have read and consented to the changes of our revised manuscript.

Finally, we should express our great thanks to you, other editors and reviewers for your helpful comments.

Yours sincerely

**Weidong Han**, Ph.D., M.D.

Department of Molecular Biology

School of Life Sciences

Chinese PLA General Hospital

Beijing 100853, P. R. China

Tel: +86-10-6693-7463

Fax: +86-10-6693-7516

E-mail: hanwdrsw69@yahoo.com

**Dear reviewers:**

Firstly, all authors should express great thanks for your positive evaluation for our manuscript. The comments you raised are very instructive, and very important for our revision. We would like to fully accept your comments and have revised our manuscript as the following. According to your comments, I have given a point-by-point response as the following.

**Your comments:**

*The review by Xiang Li et al is a comprehensive and well organized review on the current knowledge regarding the role of miRNAs in immune-response to colorectal cancer. The paper is well written and only some little details should be addressed: - Please, to complete the background of immunotherapy in CRC include in the introduction the works by Correale P et al on chemoimmunotherapy and lymphocytes infiltration in metastatic CRC patients. - when you talk about miR-484 and microsatellite instability, please also refer to the work by Le D of 2015 on the NEJM regarding the increased response of MMR deficient CRC patients to anti-PD1 therapy - what about miR-17-92 cluster? oncogene or immune activator? Please disclose better this point, considering that the cluster is upregulated by MYC and that high miR17 levels correlate with short. - please underline that cancer therapy with mir-34a (MRX34) is currently under clinical investigation (phase1) and long lasting response have been reported for solid tumors, in particular in "classical" immune-treated tumors such as renal cancer and melanoma - Give some comments on the role of miRNA in Th1 and NK response and something more on microbiota/inflammasome that is emerging as an important driver of cancerogenesis and immune-drug resistance - A further picture that better explain the role of miRNAs at different levels of the immune response is appreciated.*

**Reply:**

I deeply appreciate your positive comments and good suggestions. The comments you raised are very instructive, and very important for our revision. Frankly speaking, revision according to your comments will make our manuscript more reasonable. We have read your instructive comments very carefully and made appropriate revisions.

According to your suggestions "Please, to complete the background of immunotherapy in CRC include in the introduction the works by Correale P et al on chemoimmunotherapy and lymphocytes infiltration in metastatic CRC patients." in your comments, we added the sentences **"Moreover, immunotherapy, such as monoclonal antibody cetuximab, has immune-modulating effects in combination with traditional approaches, such as chemotherapy, thus resulting in increase of circulating dendritic cells (DCs), natural killers (NK), central memory T cells and T-helper 1 (Th1) cells[9]."** and **"However, a higher Tregs infiltration scores are correlated with overall survival, treatment-relative survival and progression-free**

**survival. Therefore, higher Tregs infiltration score is a favorable prognostic factor in advanced colon cancer patients undergoing chemo or chemoimmunotherapy[13].**" in our revised manuscript "**Introduction**" section. (**Revised portion are in red**)

According to your suggestions "when you talk about miR-484 and microsatellite instability, please also refer to the work by Le D of 2015 on the NEJM regarding the increased response of MMR deficient CRC patients to anti-PD1 therapy." in your comments, we added the sentences "**It seems to suggest that MSI is detrimental to immunotherapy for CRC treatment. MSI is the molecular feature of a mismatch repair deficient (dMMR) system[79]. Interestingly, the immunotherapy, PD-1 blockade is more effective against dMMR tumors[80]. The reason may be that the increased number of mutation-associated neoantigens in dMMR tumors stimulated antitumor immune responses.**" in our revised manuscript "**MiRNAs regulate stromal cells**" section. (**Revised portion are in blue**)

According to the suggestions "what about miR-17-92 cluster? oncogene or immune activator? Please disclose better this point, considering that the cluster is upregulated by MYC and that high miR17 levels correlate with short." in your comments, we changed "The upregulation of c-myc, the target of the miR-17-92 cluster, promotes the adenoma to adenocarcinoma transition[39]." in our previous manuscript to "**MiR-17-92 cluster increased expression during adenoma to adenocarcinoma is associated to the transcriptional activity of c-myc, which induces the expression of miR-17-92[41].**" in our revised manuscript "**immunotherapy-involved miRNAs in CRC cells**" section. (**Revised portion are in purple**) We also discussed the roles of miR-17-92 cluster in different cell types. We added the sentences "**MiR-17-5p and miR-20a increased expression, which are the member of miR-17-92 cluster, can promote tumor development in cancer cells. However, miR-17-92 members block the immunosuppressive function of MDSCs by silencing STAT3 expression. These results indicate that the functional diversity of miR-17-92 cluster may result from the different targets subjected to miR-17-92 post-transcriptional silencing in different cell types, different developmental or physiological contexts[67].**" in our revised manuscript "**MiRNAs regulate stromal cells**" section. (**Revised portion are in purple**)

According to your instructive suggestions "please underline that cancer therapy with mir-34a (MRX34) is currently under clinical investigation (phase1) and long lasting response have been reported for solid tumors, in particular in "classical" immune-treated tumors such as renal cancer and melanoma." in your comments, we added the sentences "**Actually, a miR-34 mimic (MRX34) has become the first miRNA to enter phase I clinical trial**

**(NCT01829971). The study was started to evaluate the safety of MRX34 in patients with unresectable primary liver cancer or other selected solid tumors, such as renal cell carcinoma, non-small cell lung cancer and melanoma. Targeted therapy and immunotherapy, such as PD-1 antibodies, in melanoma have led to a marked improvement in patients' survival and their quality of life[124].**" in our revised manuscript "**Perspectives and challenges**" section. (**Revised portion are in orange**)

According to your good advice "Give some comments on the role of miRNA in Th1 and NK response and something more on microbiota/inflammasome that is emerging as an important driver of cancerogenesis and immune-drug resistance." in your comments, we reviewed the roles of miRNAs in Th1 and NK cells responses. We added the sentences **"In addition to CTLs, miRNAs can regulate other immune cells, such as Th1 cells and NK cells. The members of miR-17-92 cluster miR-17 and miR-19b are the important regulators modulating Th1 responses through multiple coordinated biologic processes[30]. Moreover, miR-29 suppresses Th1 responses through the combined direct suppressive effect on T-bet, Eomesodermin and interferon γ (IFN-γ)[31, 32]. NK cells play critical roles in the innate immune system, contributing to the early detection and destruction of transformed cells[33]. MiRNAs have critical roles in controlling NK cell activation, survival and function[34]. Recent report has demonstrated that ovarian tumor-associated miR-20a decreases NK cells cytotoxicity by directly repressing MHC class I chain-related molecules A and B expression[35]."** in our revised manuscript "**Introduction**" section. (**Revised portion are in green**) We also discussed the relationship of miRNA and microbiota/inflammasome. We added the sentences **"Intestinal disease IBD and CRC are related to dysregulation of the intestinal immune system and of the microbiota[127]. The gut microbiota provides constant immunological signals to intestinal tissues, thus a variety of regulatory mechanisms have evolved to ensure proper development and function of the intestinal immune system. MiRNAs have been demonstrated to regulate expression of genes involved in microbial recognition and downstream immune activity. Thus, miRNAs play a key role within the intestinal immune system during its interactions with the gut microbiota[128]. Nine miRNAs are differentially expressed in colon of germ-free mice colonized with the microbiota; the upregulation of miR-665 significantly downregulates the ATP-binding cassette sub-family C member 3 (ABCC3)[129], which belongs to the multidrug resistance-associated protein family[130]. MiR-146a represses subset of gut barrier and inflammatory genes and restricts the expansion of intestinal T cell populations, including Tregs, Th17 and T follicular helper (Tfh) cells[131]. Furthermore, the microbiota regulation of miRNAs expression and on the maintenance of intestinal homeostasis has been investigated. The**

**expression of miR-10a and its targets IL-2/IL-23p40 play important roles in regulating innate immune responses to commensal bacteria in DC[132]. These studies open new perspectives for the investigation of miRNAs regulation in intestinal diseases and microbiota. Furthermore, a variety of bacterial infection can trigger inflammasome formation and activation[133]. Inflammasomes are large cytosolic protein complexes that promote immediate inflammatory responses and regulate intestinal homeostasis through its effects on the intestinal microbiota[134, 135]. The inflammasomes have potentially critical roles in the development and pathogenesis of IBD, because their important role in intestinal immunity[135]. Emerging evidence has demonstrated that inflammasomes can influence intestinal microbiota. Some reports have exhibited that mice lacking various components of the inflammasome lead to prototypical alterations in their microbiota, predisposing these mice to the development of IBD[136]. Inflammasomes have important roles in IBD, whereas the mechanisms of inflammasomes formation and activation in the onset of intestinal diseases have not yet been fully established. MiRNAs may be regulators in inflammasomes formation and activation. Although only few reports explore the role of miRNAs in modulating inflammasomes in intestinal diseases, some works have been carried out in other disease types[137, 138]. In the future, more investigations should illustrate novel understandings on miRNAs and inflammasomes interactions, which will potentially lead to the discovery of new treatment approaches for intestinal disease or CRC."** in our revised manuscript "MiRNAs regulate immune responses" section. (**Revised portion are in green**)

According to your suggestions "A further picture that better explain the role of miRNAs at different levels of the immune response is appreciated." in your comments, we added a more picture to explain the roles of miRNAs in CRC tumor microenvironment in our revised manuscript.

I cannot confirm whether these changes are completely in line with your requirement. If not, please let me know and give me more suggestion.

**Weidong Han**, Ph.D., M.D.

Department of Molecular Biology

School of Life Sciences

Chinese PLA General Hospital

Beijing 100853, P. R. China

Tel: +86-10-6693-7463

Fax: +86-10-6693-7516

E-mail: hanwdrsw69@yahoo.com