Dear Editor of World Journal of Gastrointestinal Oncology

Authors would like to thank reviewers for their careful review of our manuscript (No.55488) and providing us with their comments and suggestions to improve the quality of the manuscript. We have carefully considered these comments and revised the manuscript accordingly. We hope that the manuscript has now been improved by these revisions and hope that it is suitable for publication in WJGO. Point-by-point responses to comments are illustrated as following.

With best regards

Ebrahim Eftekhar, Molecular Medicine Research Center, Hormozgan Health Institute, Hormozgan University of Medical Sciences, Bandar Abbas, Iran

Reviewer #1:

Comments: This is an appreciable review, well presented and written in good English. The biggest problem is the length of the text and the repetitiveness of terms and concepts. The length of the text must be reduced by 20% the paper is too long and in some points repetitive. Introduction and conclusions should be shortened. Many sentences of the text are well known and do not represent any interesting news. It would be better to reduce the length of the work. it would be much better to stress the major and most recent innovations in resistance training considering only the biomolecular genetics studies of the last 5 years.

Author's response: Dear reviewer thanks for your valuable suggestions. Some redundant and repetitive sentences were omitted from all part of the manuscript including ''Introduction section'' and Conclusion. The deleted sentences are listed below. Some other sentences were changed in text and were highlighted in yellow. In the present manuscript we focus on the most important and the most relevant molecular factors that affect 5-FU drug response in colorectal cancer. Microsatellite instability, increased expression level of thymidylate synthase and dihydropyridine dehydrogenase, mutation of *TP53* as well as miRNAs alterations are among the main molecular determinants of response to 5-flurouracil-based chemotherapy in CRC. In the last 3 years the role of circular RNA in 5-FU resistance in CRC was also discussed. Although few studies are available in this regard, we added a new subtitle "Circular RNA and resistance to 5-FU" to the manuscript. Certainly circular RNA could be an important molecular determinant of response to 5-FU drug in CRC in the near future.

Some sentences or parts that have been deleted from "introduction section" are as follow:

-accounting for 881,000 deaths in 2018

-radiotherapy, immunotherapy, and targeted therapy

-and several other common malignancies such as breast, esophageal, and gastric cancers

-patients and a five-year survival rate for mCRC is around 12 percent. Approximately 50 percent of mCRC patients show resistance to 5-FU-based chemotherapies

The subtitles 'Chemotherapy in colorectal cancer' and "Fluorouracil-based chemotherapy in colorectal cancer" were merging and presented as new subtitle: Fluorouracil-based chemotherapy and colorectal cancer. Some sentences that were deleted from these two parts including:

- Until today, the systemic chemo treatment of CRC has been based on two different lines. The first-line chemotherapy with palliative regimen predominantly includes fluoropyrimidines (e.g., 5-FU or capecitabine) alone or in combination with LV as well as other cytotoxic agents, such as irinotecan [5-FU/LV/irinotecan (FOLFIRI)], OXA [5-FU/LV/OXA (FOLFOX) and capecitabine/LV/OXA (CAPOX)]. Capecitabine is one of the prodrugs that have been developed from 5-FU. This orally-administered fluoropyrimidine carbamate is preferably converted to 5-FU within the tumor region [12]. The second-line chemotherapy is widely recognized as a line of treatment which is based on a refractory-based regimen. For patients who are resistant to irinotecan, the treatment will consist of an OXA-containing combination such as CAPOX or FOLFOX, whereas patients who are resistant to CAPOX or FOLFOX will be treated with FOLFIRI or irinotecan monotherapy [13].

- This drug was first introduced in 1957, following its favorable effect on malignant over nonmalignant cells

- administered intravenously [17, 18]. The structural formula of this heterocyclic aromatic organic compound is similar to uracil except for containing a fluorine atom at the 5-carbon position on the ring. 5-FU molecular weight is 130 Dalton [19].

In subtitle "Mechanism of 5-FU action" some sentence were also deleted:

- Subsequently, some important in vivo as well as in vitro findings have documented that 5-FU treatment mediated triggered programmed cell death, which is the main event in the killing of human gastric and colon tumors. TS inhibition may activate programmed cell death through the 'genotoxic stress' pathways, leading to the induction of parental DNA fragmentation. The genotoxic stress phenomenon, resulting from TS inhibition can activate the overexpression of the cellular oncoproteins such as bcl2 and mutant *TP53* [24].

In subtitle "Mechanism of 5-FU resistance" some sentence were also deleted:

-5-FU resistance is responsible for a great proportion of mCRC-related death, limiting the effectiveness of current cancer therapies, including those used to treat CRC.

-Since even the combination of 5-FU with other anti-tumor drugs has merely improved the response rates only to 40–50%, there is an urgent need for developing new strategies for therapy and resistance counteract..

- Furthermore, drug-resistant micrometastatic tumor cells are likely to reduce the effectiveness of adjuvant chemotherapy following surgery

The subtitle "MicroRNA biosynthesis and its function in cancer progression and 5-FU drug resistance in colorectal cancer" was changed to "MicroRNAs function in cancer progression and 5-FU drug resistance in colorectal cancer" and some sentences that were deleted from this section are as follow:

-Drosha and Dicer are 2 main enzymes that play an important role in converting Pri and Pre-miRNA to a mature miRNA [31, 33]. Mature microRNA acts as a part of the RNA-induced silencing complex (RISC) which can target and bind specific mRNAs [31, 32]. Only matching a small part of miRNAs, which are termed as a seed region, with a base sequence is usually enough for being recognized as a target for the RISC complex [31]. So, a specific miRNA can have more than one target as the seed region may bind to more than one specific sequence [31]. Therefore, deregulation of a specific miRNA may disrupt a sequence of genes and pathways rather than only one specific gene function.

-Even more, overexpression of specific oncogenic miRNAs can develop because of epigenetic changes during cancer. Hypomethylation of miRNAs' promotor region will affect their expression, resulting in overexpression of oncogenic miRNAs

- Besides, some other functional miRNAs are discussed in two distinct sections referred to ''the miRNAs promote 5-FU drug resistance in CRC'', and ''the miRNAs reverse 5-FU drug resistance in CRC''.

In subtitle"Thymidylate Synthase (TS) and resistance to 5-FU" some sentences were deleted:

-Moreover, thymidine kinase can lessen the effects of TS deficiency by the salvation of thymidylate from thymidine. This salvage pathway indicates a potential mechanism of resistance to 5-FU

- Therefore, the increased likelihood of survival of cancer cells treated with 5-FU in TSER*3/TSER*3 patients might be due to higher levels of TS expression in the tumors of these patients compared with patients with the TSER*2/TSER*2 and TSER*2/TSER*3 genotypes

- The expression of various miRNAs has been related to 5-FU response in the literature and the target of some of these miRNAs is TYMS

In subtitle" Dihydropyrimidine dehydrogenase (DPD) and resistance to 5-FU" some sentences were deleted:

- Surprisingly, the oral 5-FU prodrug capecitabine avoid DPD-mediated degradation in the liver

- Moreover, Bai and colleagues reported that increased levels of these three prognostic markers are in association with increased 5-FU chemoresistance and poorer survival rates

In conclusion section some sentences were deleted:

Since CRC remains one of the most severe and fatal cancer diseases, and the reason is mostly rooted in the chemoresistance phenomenon, we decided to put our efforts to comprehensively review the current issues regarding the molecular mechanisms involved in 5-FU drug resistance in CRC

Results of different studies indicated that TSER*3/TSER*3 homozygous patients are less likely to respond to 5-FU-based chemotherapy than TSER*2/TSER*2 homozygous and TSER*2/TSER*3 heterozygous patients

Regarding 5-FU treatment, Nakajima et al. demonstrated that assessment of miR-181b and let-7g in patients who receive S-1, the fourth-generation of an oral 5-FU drug, could predict the response rate to S-1-based chemotherapy [93]. Thereafter, we determined to focus on the roles of miRNAs in 5-FU resistance in CRC. We could review around 40 different miRNAs, with detailed information regarding their targets and other main findings (table 1). We also performed bioinformatics analysis using DIANA-miRPath v3.0, to complete table 1. Surprisingly, many of studied miRNAs were involved in common signaling pathways including proteoglycans in cancer, fatty acid metabolism, cell cycle, adherent junction, ECM-receptor interaction, hippo signaling pathway, TGF-beta signaling pathway and signaling pathways regulating pluripotency of stem cells, some of them are in tight association with CRC.

Additionally in order to decrease the length of table 1, some references (9 references) were deleted. The list of deleted references was:

1-Jin Y, Jiang Z, Guan X, Chen Y, Tang Q, Wang G, Wang X. miR-450b-5p Suppresses Stemness and the Development of Chemoresistance by Targeting SOX2 in Colorectal Cancer. *DNA Cell Biol* 2016; **35**: 249-56 [PMID: 26845645 DOI: 10.1089/dna.2015.3120]
2-Wang L, Jiang CF, Li DM, Ge X, Shi ZM, Li CY, Liu X, Yin Y, Zhen L, Liu LZ, Jiang BH. MicroRNA-497 inhibits tumor growth and increases chemosensitivity to 5-fluorouracil treatment by targeting KSR1. *Oncotarget* 2016 19; **7**: 2660-71 [PMID: 26673620 DOI: 10.18632/oncotarget.6545]
3-To KK, Leung WW, Ng SS. Exploiting a novel miR-519c-HuR-ABCG2 regulatory pathway to overcome chemoresistance in colorectal cancer. *Exp Cell Res* 2015 1; **338**: 222-31 [PMID: 26386386 DOI: 10.1016/j.yexcr.2015.09.011]

4-Li X, Li X, Liao D, Wang X, Wu Z, Nie J, Bai M, Fu X, Mei Q, Han W. Elevated microRNA-23a Expression Enhances the Chemoresistance of Colorectal Cancer Cells with Microsatellite Instability to 5-Fluorouracil by Directly Targeting ABCF1. *Curr Protein Pept Sci* 2015; **16**: 301-9 [PMID: 25929864 DOI: 10.2174/1389203716041504291533309]
5-He J, Xie G, Tong J, Peng Y, Huang H, Li J, Wang N, Liang H. Overexpression of microRNA-122 re-sensitizes 5-FU-resistant colon cancer cells to 5-FU through the inhibition *of PKM2 in* vitro and in vivo. *Cell Biochem Biophys* 2014; **70**: 1343-50 [PMID: 24898807 DOI: 10.1007/s12013-014-0062-x]
6-Shang J, Yang F, Wang Y, Wang Y, Xue G, Mei Q, Wang F, Sun S. MicroRNA-23a antisense enhances 5-fluorouracil chemosensitivity through APAF-1/caspase-9 apoptotic pathway in colorectal cancer cells. *J Cell Biochem* 2014; **115**: 772-84 [PMID: 24249161 DOI: 10.1002/jcb.24721]
7-Siemens H, Jackstadt R, Kaller M, Hermeking H. Repression *of* c-Kit by p53 *is* mediated by miR-34 and is associated with reduced chemoresistance, migration and stemness. *Oncotarget* 2013; **4**: 1399-415 [PMID: 24009080 DOI: 10.18632/oncotarget.1202]
8-Karaayvaz M, Zhai H, Ju J. miR-129 promotes apoptosis and enhances chemosensitivity to 5-fluorouracil in colorectal cancer. *Cell Death Dis* 2013 6; **4**: e659 [PMID: 23744359 DOI: 10.1038/cddis.2013.193]
9- Nishida N, Yamashita S, Mimori K, Sudo T, Tanaka F, Shibata K, Yamamoto H, Ishii H, Doki Y, Mori M. MicroRNA-10b is a prognostic indicator in colorectal cancer and confers resistance to the chemotherapeutic agent 5-fluorouracil in colorectal cancer cells. *Ann Surg Oncol* 2012; **19**: 3065-71 [PMID: 22322955 DOI: 10.1245/s10434-012-2246-1

Reviewer#2:

Comments: Authors should carefully check the accuracy of the language and abbreviations of this manuscript. For example, "Moreover, activation of the RAS/AKT/PI3K signaling pathway by overexpression of miR-224 can provide a similar effect of F-5U resistance."(see on page 22)

Author's response: Dear reviewer thanks for your valuable suggestion. The manuscript was edited for the second time by a person who has PhD degree in English literature. The Certificate of second round proofreading was attached. All the abbreviations were double checked. This sentence "Moreover, activation of the RAS/AKT/PI3K signaling pathway by overexpression of miR-224 can provide a similar effect of F-5U resistance" was also changed and highlighted in yellow (page 12)

Editorial Office's comments

***Science Editor*:** The authors did not provide original pictures. Please provide the original figure documents. Please prepare and arrange the figures using PowerPoint to ensure that all graphs or arrows or text portions can be reprocessed by the editor.

Author's response: Thanks for your valuable suggestion. The original figures in PPT format were uploaded.