Dear editor and reviewers:

" The function and molecular mechanisms of resveratrol in the prevention and treatment of gastrointestinal cancer: A review" by Liyan Wang *et al* (World Journal of Clinical Cases Manuscript NO: 53582)

Thank you for sending us the referees' comments and giving us valuable suggestions. We have detailed replies point-by-point to each reviewers' comments. We have also made necessary corrections and supplied the relevant examples in accordance with the suggestion of the reviewers. We highlight the changes made in our manuscript with the underline tool, and the revised manuscript has been uploaded to the website of **World Journal of Clinical Cases**. We hope the revised manuscript can satisfy you and the referees and meet the high standard required for the journal.

Sincerely yours,

Liyan Wang

Department of Pharmacy，the First Affiliated Hospital of Dalian Medical University, Dalian, 116011, China

Tel. /fax: +86-0411-83634643

E-mail: wangliyan3626@126.com

Response to Reviewer 2's Comments:

Referee:2

1. **Though it is an interesting review, the manuscript is very wordy and difficult to read. Polishing and better synthesis of the overall findings are needed. Many unnecessary details and statements would have to be stripped away to ease the reading of the manuscript.**

Response: Thank you very much for your comments and kindly suggestion. We have polished carefully, and do some appropriate synthesis of the manuscript, many unnecessary details and statements have been stripped away. We highlight the changes made in our manuscript with the underline tool. And we also highlight the details that been stripped away with the strikethrough tool as following.

According to the ~~2018~~ Global Cancer Statistics[1], CRC is the second leading cause of cancer-related mortality, closely followed by GC. GC ~~(cardia and noncardia gastric cancer combined)~~ was responsible for over one million new cancer cases in 2018 and an estimated 783,000 deaths, which equates to 1 in every 12 deaths globally. ~~Incidence rates are two-fold higher in men than women and are markedly elevated in the Eastern Asian population.~~ Over 1.8 million new CRC cases and 881,000 deaths were estimated to occur in 2018, ~~accounting for about 1 in 10 cancer cases and deaths. China has experienced increases in both the incidence of CRC and associated mortality over the last decade[2].~~

Moreover, many studies have demonstrated that Res might also exert a chemopreventive effect when combined with other chemotherapeutic drugs. To date, the various anticancer molecular mechanisms of Res in the prevention and treatment of gastrointestinal cancer have implicated multiple pathways in its effects, including oxidative stress, cell proliferation, and apoptosis ~~have been studied, including the antioxidant, anti-inflammatory, anti-proliferative, and anti-angiogenic mechanisms[9]. Further, studies of the anticancer mechanisms of Res in the prevention and treatment of gastrointestinal cancer have implicated multiple pathways in its effects, including oxidative stress, cell proliferation, and apoptosis.~~

1 Gastric (stomach) cancer

1.1 Resveratrol as a preventive agent against Helicobacter pylori (H. pylori)

Constant overproduction of nitric oxide may lead to DNA and tissue damage,

ultimately increasing the risk of developing cancer[28]. ~~It has been shown that~~ The activation of the NF-κB signal transduction pathway is also known to be an important event linked to tumorigenesis[23]. ~~NF-κB can be activated by ROS. H. pylori-induced oxidative stress has also been investigated in a mouse model with oral inoculation of H. pylori[24]. Mice administered Res (100 mg/kg /day) orally for six weeks were found to exhibit down-regulation of protein expression levels of IL-8 and iNOS, which are regulated by NF-κB, suppression of H. pylori-induced phosphorylation of IκBα (which embodies the level and activation of NF-κB), and increased levels of HO-1（a potent antioxidant enzyme） and Nrf2. The results suggest that~~t Res exerts significant effects against oxidative stress and inflammation by way of suppressing expression levels of IL-8 and iNOS, blocking the activation of NF-κB, and activating the Nrf2/HO-1 pathway.

1.3 Inhibition of cancer cell invasion and metastasis

Cancer cell invasion and metastasis are interconnected processes that involve cell proliferation, cell migration, cell adhesion, and proteolytic degeneration of tissue barriers ~~such as the extracellular matrix (ECM) and basal membrane. Numerous studies have indicated that Res suppresses cancer cell invasion and metastasis in several cancers[36].~~ GC exhibits high motility due to its strong invasion and metastasis ability. The hedgehog (Hh) signaling pathway plays an important role in vertebrate development, the homeostatic process, and tumorigenesis. ~~Both the Hh signaling pathway and epithelial-mesenchymal transition (EMT) are cellular processes associated with cancer metastasis and invasion. Treatment of gastric cancer SGC-7901 cells with~~ Res suppresses invasion and metastasis via inhibition of the Hh signaling pathway and epithelial-mesenchymal transition (EMT) which is also associated with cancer metastasis and invasion. Res ~~at various concentrations~~ was found to produce decreased expression of Gli-1, a key component of the Hh signaling pathway, as well as decreased expression of Snail and N-cadherin and increased expression of E-cadherin, ~~each of~~ which are key components of EMT [37]. ~~These findings indicate that Res suppresses invasion and metastasis of SGC-7901 cells via inhibition of the Hh signaling pathway and EMT.~~ In addition, Res could inhibit metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) expression [42].

Interleukin-6 (IL-6) is a multifunctional cytokine that plays a vital role in host defense mechanisms and growth of various cancer cells[38]. Yang found that ~~a~~

non-cytotoxic concentration（20 μmol/L）of Res inhibited IL-6-induced cell invasion and matrix metalloproteinases activation by blocking Raf/MAPK signaling activation in SGC7901 cells[39].

A recent study reported that Res suppresses invasion and migration through inhibition of MALAT1-mediated EMT[40]. Treatment of the human gastric cancer cell line BGC823 with 200 μmol/L Res significantly suppressed cell migration and invasion. Additionally, Res inhibited metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) expression. When MALAT1 was knocked down, cell viability, migration, invasion, and EMT were inhibited[40].

1.5 Resveratrol reverses the multidrug resistance (MDR) of cancer cells

Chemotherapy is considered to be the most effective treatment for patients with inoperable cancers; it provides palliative treatment of symptoms and improves patient quality of life and survival. However, conventional chemotherapeutic drugs such as doxorubicin and apatinib have been criticized due to their negative effects, including the development of drug resistance and the occurrence of tumors[55,56]. Xu et al. found that Res could reverses Doxorubicin (DOX) resistance by reversing the EMT process via modulation of the PTEN/Akt signaling pathway[62]. Xu et al. established a Doxorubicin (DOX)-resistant human gastric cancer cell line (SGC7901/DOX) derived from SGC7901 cells and exposed this cell line to stepwise increasing concentrations of DOX. Treatment with 50 mg/L Res enabled SGC7901/DOX cells to regain DOX sensitivity, mitigated the aggressive biological features, and promoted cell apoptosis *in vitro*. Res treatment at 50 mg/kg also inhibited tumor growth in nude mice bearing subcutaneous SGC7901/DOX xenograpfts[57]. Mechanistic studies have revealed that Res reverses DOX resistance by reversing the EMT process via modulation of the PTEN/Akt signaling pathway.

2. **Colorectal cancer**

2.1 Resveratrol as an anti-inflammatory agent in CRC

Many studies have demonstrated that Res has a promising preventive and therapeutic effect on CRC *in vitro* and *in vivo*. COX enzymes (COX-1 and COX-2) catalyze the conversion of arachidonic acid to pro-tumorigenic eicasonoids such as prostaglandin E-2 (PGE-2), which are involved in the maintenance of the malignant phenotype. COX-2 emzyme expression is usually upregulated during inflammation in most human adenocarcinomas and colonic tumors, and in ApcMint mouse

adenomas[59,60,61]. In one study, ApcMint mice, which represent a model of human intestinal carcinogenesis, received a Res dietary concentration of 0.2% (approximately 240 mg/kg Res) and the results revealed that adenoma load was decreased by 27%[62]. Further, incubation of HCA-7 cancer cells for 24–96 hours with a stilbene derivative (1–50 μmol/L) containing Res decreased COX-2 protein expression in CRC cell [67]. Chronic colitis is associated with colon cancer risk and Res mixed with food was found to ameliorate a dextran sulfate sodium (DSS) mouse model of colitis in a dose-dependent manner[63]. Further, the inflammation score was markedly increased, the percentage of neutrophils in the mesenteric lymph nodes and lamina propria was decreased, and CD3+ T cells that express tumor necrosis factor-α and interferon factor-γ were decreased [63]. In addition, Res was found to downregulate p53 and p53-phospho-serine 15, which are markers of inflammation and inflammatory stress[63]. Res can also reverse downregulation of SIRT-1 during colitis, which It is known that SIRT-1 may induce inflammatory cytokines through the activation of NF-κB; Hofseth and his team found that Res can also reverse downregulation of SIRT-1 during colitis. Hofseth et al. believe this to be an important anti-inflammatory mechanism of Res[64]. Lipopolysaccharide (LPS), a principal component of the outer membrane of Gram-negative bacteria, plays a critical role in triggering an early inflammatory response. Res reduces the LPS-induced inflammatory response through interfering with LPS-induced NF-κB activation[70].Panaro et al. evaluated the effect of Res on inflammatory responses induced by LPS treatment in human intestinal Caco-2 and SW480 cell lines. Res inhibited the expression of inducible NO synthase (iNOS) mRNA as well as protein expression, resulting in a dose-dependent decrease in the production of NO. Res also inhibited phosphorylation and degradation of the inhibitor of κB (IκB) complex. These results indicate that Res reduces the LPS-induced inflammatory response through interfering with LPS-induced NF-κB activation[65]. Nuclear factor erythroid 2 related factor 2 (Nrf2) is recognized as a drug target for the prevention of CRC. Mitogen-activated protein kinase phosphatase 1 (Mkp-1) interferes with the Nrf2 signaling pathway, protecting against intestinal inflammation. Li et al. demonstrated the role of the Mkp-1/Nrf2 axis in limiting inflammation in DSS-induced murine colitis[66]. In colitis-associated tumorigenesis mice, Mkp-1−/− mice exhibited a phenotype similar to Nrf2−/− mice, with significantly more tumors than BALB/c background wild-type (WT) mice[67]. Tumors from Mkp-1−/− mice exhibited

~~over-expression of the nitrotyrosine marker of oxidative stress and the 53BP1 marker of DNA damage, respectively [67]. Moreover,~~ Res ~~(300 pmol/L)~~ supplementation suppressed tumorigenesis in colitis-associated tumorigenesis mice ~~in WT but not in Mkp-1−/− mice. Dd~~own-regulation of Nrf2 and its target genes were observed in adenomas from adjacent normal tissue. ~~These results indicate that Mkp-1 plays a protective role of Nrf2 signaling against colitis-associated tumorigenesis[67].~~

FUTURE OUTLOOK

Despite all of the documented potential anticancer effects of Res in cell culture models and animal models, we cannot assume that the potential properties of Res ~~in cell culture or animal models~~ can be translated to humans because of the low bioavailability of Res ~~in humans~~. Due to the rapid metabolized and glucuronidation and sulfation in intestine and liver ~~Early research revealed that~~ the bioavailability of Res in humans was considerably less than 1%[113,114] despite high absorption of almost 70%~~; this is due to its rapid and extensive metabolism involving both glucuronidation and sulfation in the intestine and liver[113,114].~~ The poor bioavailability of Res is an important problem in terms of extrapolation of its potential clinical application ~~effects to humans.~~ Several ~~Different~~ methods have been developed to improve its bioavailability ~~in humans,~~ such as utilizing it in combination with an additional phytochemical curcumin or using nanotechnological formulations[115,116,117,118].

Yu et al reported that combined treatment with curcumin and Res in dimethylhydrazine (DMH)-treated ~~rats, a~~ gastric cancer rats ~~model,~~ They found ~~resulted in a significant decrease in tumor burden. Further,~~ the combination regulated both p53 phosphorylation and acetylation so as to produce stable tumor suppressor p53 against gastric cancer[119]. Gavrilas ~~and his team~~ also ~~reported~~ investigated cell proliferation inhibit effect of ~~that~~ the combined ~~treatment~~ of curcumin and Res ~~inhibited cell proliferation in a dose-dependent manner,~~ they found the ~~exerting a~~ synergistic effect ~~in~~ both in ~~the~~ DLD-1 cells ~~line~~ and ~~the~~ Caco-2 cells ~~line~~. The expresser of several genes involved in the modulation of apoptosis, including PMAIP1, BID, ZMAT3, CASP3, CASP7, and FAS was significantly increased ,represent new targets for combined treatments with curcumin and Res[121-122].

Lipid-core nanocapsules (LNCs) have also been reported to stabilize the incorporated drugs, control their release pattern, and ~~improve their therapeutic performance, and ultimately,~~ increase the activity of the drugs in the body[121]. Xiong

et al. prepared Res-loaded LNCs (RSV-LNCs) and found that RSV-LNCs exerted a remarkable reduction in cell apoptosis of ~36%. This suggests that they have a superior anticancer effect and promising potential to increase the therapeutic efficacy of Res[122].

CONCLUSION

Gastrointestinal cancer, particularly CG and CRC, is a highly prevalent cancer and one of the leading causes of cancer-related deaths worldwide. ~~Several factors, including family history and lifestyle factors, are involved in the incidence of this malignancy.~~ Multiple molecular mechanisms ~~and various signaling pathways,~~ including inflammation, oxidative stress, cell proliferation, and apoptosis, are associated with its incidence and progression[123]. Res is a polyphenol compound with varied properties, including anti-inflammatory, cell proliferation inhibition, and apoptosis properties[124,125]. Multiple studies have demonstrated the potential effects of Res for the treatment of GC and CRC. This phytochemical has multiple advantages, has been shown to be comparatively safe, and is able to target multiple cell signaling pathways. However, the bioavailability of Res is very low in humans ~~because of its metabolic characteristics,~~ and a high dose ~~of Res~~ may not reach a sufficient concentration of treatment requir~~ed for systemic treatment of cancers[126].~~ due to the metabolic characteristics [129].Res may be of benefit for digestive tract cancer treatment as it is efficiently absorbed in the gastrointestinal tract and exerts local effects before its metabolization[127,128]. ~~In addition, various methods have been developed to improve the bioavailability of Res. This phytochemical has multiple advantages, has been shown to be comparatively safe, and is able to target multiple cell signaling pathways. As such, Res is considered to be a promising preventive and therapeutic agent for cancers. In particular, this phytochemical is a promising candidate for gastrointestinal cancer prevention and treatment. Nonetheless,~~ Although various methods have been developed to improve the bioavailability of Res, more studies are needed to verify the efficacy of Res in gastrointestinal cancer. ~~in humans.~~

2. The title section 2.2 “Resveratrol inhibits oxidative stress in CRC” is particularly misleading since the authors comment on studies showing that resveratrol can lead to elevation of ROS production. Specific sections describing data obtained in animal models would be helpful.

Response: Thank you very much for your comments. We have stripped away the

example which seems to be misleading and rewrite this section. We add one animal models with describing data which would support our point. We highlight the changes with an underline tool in revised manuscript. The details were as following.

2.2 Resveratrol inhibits oxidative stress in CRC

Colon cancer is known to be a pathological consequence of persistent oxidative stress, resulting in mutations and DNA damage in cancer associated genes in which the cellular overproduction of ROS is implicated [73,74]. Nalini et al demonstrated that 1,2-dimethylhydrazine (DMH) induced DNA damage and oxidative stress were suppressed effectively by chronic Res supplementation. Particularly, entire-period Res supplementation increased the enzymic (glutathione reductase, superoxide dismutase, glutathione peroxidase ,catalase and glutathione S-transferase) and non-enzymic (reduced glutathione, vitamin C, vitamin E and β -carotene) antioxidant status with a corresponding decrease in the extent of lipid peroxidation markers such as thiobarbituric acid reactive substances, diene conjugates and lipid hydroperoxides[74].

3. Additionally, We placed One hundred and twenty-seven references were cited, including thirty-eight references published in the last five years and fourteen references are self-citation. We highlight the changed references in our manuscript with the underline tool. We checked the manuscript carefully and removed the un-academic language.