Dear professor Tang,

Thank you for the editor and the reviewer providing us for the conscientious and insightful review to improve the quality of manuscript. We read a large number of literature, including Epidemiol 2016 and Annu Rev Pathol 2019 and so on,which benefits me and my team a lot. Based on what we've learned through the literature, we supplemented these issues in our manuscript. In the response below, we have addressed all the issues raised by the editor and the reviewer. All changes have been marked in blue.

We hope that our revised manuscript has been improved and meet the academic standard of the editor and reviewer. Eventually, we expect that our manuscript are suitable for publication in your magazine. Thank you very much.

Yours Sincerely,
Shitong Wang and Lixuan Sang

Response to Reviewers Comments
Manuscript NO: 52137
Title: Tea Polyphenols and Their Chemopreventive and Therapeutic Effects on Colorectal Cancer

Authors: Shitong Wang, Wenqi Cui, Dan Pan, Min Jiang, Bing Chang, Lixuan Sang

Reviewers' comments:

Reviewer:
1) This paper discuss many experimental data but lacks detailed discussion on data of diets and lifestyle influence on pathogenesis and pathologies in humans.

Response: Thank you for the pointing out the problem.We really overlooked the impact and the relative mechanism of diet and lifestyle acting on colorectal cancer.In addition to the alifestyle and tea polyphenols on colorectal cancer.We also have supplemented that.Therefore,we revised the manuscript as following.

Diet and lifestyle: CRC is generally reported as diet-and lifestyle-related pathology and is associated with several main factors: diet, physical activity, consumption of alcohol, cigarette and aspirin[7].

Diet: Findings from a systematic review have demonstrated that various of foods are associated with CRC positively or negatively[8]. In general, the higher or lower risk of CRC is related to the proinflammatory or anti-inflammatory property of the food, respectively[9]. Moreover, different foods can exert the function with a different mechanisms. And then take some of these foods as examples to explain the mechanism that they act on CRC briefly. **Red and processed meat:** There are several systematic reviews and epidemiological studies indicating that intake of red and processed meat will increase the risk of CRC[10-12]. Besides, a study demosntrated that the consumption of red and processed meat was associated more strongly with an increased risk of CRC with KRAS-wildtype, indicating that the potential mechanism should be studied[12]. Red and processed meat at high temperatures results in the formation of heterocyclic amines and polycyclic aromatic hydrocarbons, and then allows the formation of DNA adducts that

subsequently cause DNA damage to promote the tumorgenesis[13,14]. In red meat, heme is present in high concentrations in the form of myoglobin and a large amount of heme iron intake was associated with a higher risk of CRC[14]. Heme iron from red and processed meat can stimulate the metabolism of nitrate/nitrite and the formation of N-nitroso compounds and induce oxidative stress and lipid peroxidation (LPO), to trigger inflammatory response, and thereby promote the development of CRC[14,15]. **Dietary fats:** Dietary fats are also associated with CRC. A high intake of ω-6 polyunsaturated fatty acid (PUFA) and saturated fat has tumor-enhancing effects[16]. Rapid metabolism of arachidonic acid (AA), increased activities of phospholipases and the elevated levels of cyclooxygenase (COX) and lipoxygenase (LOX) may suggest the potential mechanism of fatty acid promoting the incident of CRC[17]. However, ω-3 PUFA intake can reduce the risk of CRC, particularly with microsatellite instability (MSI)-high cancer subtype or high FOXP3+ regulatory T cell (Treg cell) counts[18,19]. ω-3 PUFA exerts the effect of anticancer through several potential molecular mechanisms including suppression of arachidonic acid-derived eicosanoid biosynthesis, impact on transcription factor activity, gene expression, and signal transduction pathways, increased or decreased production of free radicals and reactive oxygen species and so on[20]. In addition, marine ω-3 PUFA also protects against CRC through inhibition of the T cell-suppressive activity of Treg cells[19]. In addition, oleic acid, the main monounsaturated fatty acid (MUFA) in olive oil also exerts a protective effect on CRC[21,22]. A competitive inhibition by oleic acid of the Δ6-desaturase will suppress the eicosanoid biosynthesis of arachidonic acid to disrupt the tumor growth progresses[21]. **Vitamin D:** Vitamin D can inhibit the development of CRC, particular some specific subtypes of CRC. The beneficial survival association of high vitamin D level is stronger for CRC with lower-level peritumoural lymphocytic reaction than for carcinoma with higher-level reaction[23]. Vitamin D is hydroxylated in the liver to produce 25(OH)D that serves as a standard indicator of vitamin D activity[23]. And then 25(OH)D is hydroxylated further in the kidneys to produce a hormonally active metabolite, 1,25-dihydroxyvitamin D[23]. Vitamin D and its metabolites exert their anti-neoplastic effect by binding with the transcription factor vitamin D receptor (VDR)[24]. Vitamin D may suppress signaling pathways and cytokines and modulate adaptive immune cells such as B cells, helper T cells (Th cells) and Treg cells[24]. Moreover, vitamin D diet can also cause significant changes in the fecal microbial community structure. During the development of CRC, Vitamin D deficiency can not only cause a sharp decrease in Akkermansia muciniphila (A. muciniphila), but also induce changes in the expression of mucus and goblet-cell associated genes, so that the gut barrier integrity is destroyed[25]. **Dietary fiber:** A high intake of dietary fiber, particularly derived from vegetables and fruit, was inversely associated with CRC risk[26,27]. This association was driven mainly by the position of the tumor, which was stronger for the risk of rectal cancer[28]. However, a new study also indicated that the relationship of fiber and risk of CRC independing on tumor subsite or molecular marker[29]. In addition, higher intake of dietary fiber was more strongly associated with lower risk for Fusobacterium nucleatum (F.nucleatum)-positive CRC, but not F.nucleatum-negative CRC[30]. The findings suggest a potential role for intestinal microbiota in mediating the association between fiber and CRC[30]. Fiber can be fermented by the gut bacteria into

short-chain fatty acids, such as butyrate, acetate, and propionate, that possess a diversity of tumorsuppressive effects[31]. High level of short-chain fatty acids producing from fiber might alter pH, increase transit time of gut contents, and lead to differences in local immune surveillance,and thereby reduce the growth of harmful species such as F. nucleatum[30]. In addition, the potential mechanism that fiber inhibiting the development of CRC also contains other aspects: increasing the stool bulk; shortening the bowel transit time; diluting the effect of potential carcinogens; and altering bile acid metabolism[26,32]. **Selenium(Se):** Epidemiological investigation has demonstranted that higher Se levels were inversely associated with the risk to develop CRC in the European[33]. Usually, dietary Se intake is essential for synthesizing selenoproteins that are important in inhibiting oxidative and inflammatory processes linked to colorectal carcinogenesis[34]. Se supply might play an important role in regulating expression of some selenoproteins such as glutathione peroxidases(GPX1), selenoprotein F (SELENOF), SELENOP, SELENOK, and components of the thioredoxin reductase system (TXNRD1-3), to reduce the oxidative stress and inflammatory response[34,35]. However, some studies also suggested that the selenoprotein expression may affect CRC development independent of the Se status, even lead to the development of CRC with suboptimal Se status[34,35]. In addition, a study also indicated that intake of Se nanoparticles (SeNPs) can activate autophagy to promote cancer cells death, through upregulation of Beclin 1-related signaling pathways[36]. **Folic acid:** Accumulating evidence displays that folic acid can also be an effective chemopreventive agent for CRC[37,38]. Supplemental folic acid has been shown to prevent the loss of heterozygosity (LOH) of the tumor suppressor gene that deleted in colorectal cancer and to stabilize its protein in normal appearing rectal mucosa of patients with colorectal adenomas[38]. In addition, deficiency folic acid may lead to inadequate purine and pyrimidine synthesis and changes in methylation, with a concomitant impact on DNA replication and cell division due to the disruption of folate cycle[39]. Thus, deficiency folic acid can promote EGFR expression through reducing methylation of CpG sequences within its promoter[38]. **Others:** Other foods are also associated with CRC positively or negatively. High sugar foods, spicy foods might have a positive association with CRC risk, however, vegetables, soy bean/soy products, seafood, vitamins C, E and B12 play a protective role against CRC risk[6-8]. These foods exert the promotive or protective effect on CRC through modulating the inflammatory response, insulin resistance, and the composition of gut microbiota mainly[40,41].

Physical activity(PA): Physical inactivity also has been well known as a lifestyle risk factor for CRC. There are many epidemiological studies indicating that PA was associated with a statistically significant reduction in colorectal cancer risk[42-45]. Moreover, the association may depend on the location of tumor and gender[42-45]. Some meta-analysis and systematic reviews indicated that PA is associated with reduced risk of both proximal colon and distal colon cancer, but the difference was observed between the colon and the rectu[42,43]. There is even no association observed between PA and rectal cancer[43]. And, gender is another factor to impact the relationship of between the PA and CRC. A systematic review has observed that an apparent interaction between sex and PA in relation to CRC risk, a statistically significant reduced risk among men, but statistically nonsignificant reduced risk among women[45]. In addition, there is a potential interactive

effect of PA and sedentary time on CRC risk. The benefits of moderate to strenuous-vigorous PA on CRC risk are observed most clearly among those with more sedentary time because these individuals have lower total activity[45]. Several plausible biological mechanisms have been proposed, including changes in endogenous sexual and metabolic hormone levels and growth factors, decreased obesity and central adiposity, and possibly changes in immune function and so on[46]. Peroxisome proliferator-activated receptor gamma coactivator 1α (PGC-1α) is a mitochondrial regulator in a wide variety of biological processes-such as thermogenesis, circadian rhythm, fatty acid oxidation, glucose metabolism, mitochondrial organization, and biogenesis[46]. PA, as a stressor that demands energy, stimulates PGC-1α expression, increasing biological processes on CRC and suppressing the development of CRC[47].

Consumption of alcohol: High consumption of alcoholic beverages may lead to the increasing risk of CRC[48,49]. Consumption of alcohol is also relative to molecular subtypes of CRC. Alcohol intake was positively related to risk of BRAF-tumors but not to risk of BRAF+tumors, irrespective of their KRAS status[50]. Similarly, a study has demonstrated that higher alcohol consumption was associated with risk of colorectal cancer with insulin-like growth factor 2 (IGF2) differentially methylated region-0 (DMR0) hypomethylation but not risk of cancer with high-level IGF2 DMR0 methylation[51]. IGF2 upregulation by DMR0 hypomethylation caused by alcohol may promote tumorigenesis in colorectal tissue[51]. Alcohol can also interfere with one-carbon metabolism, a complex network of interrelated biochemical reactions that involve the transfer of one-carbon (methyl) groups from one compound to another[52]. Excess alcohol can antagonize methyl donors including vitamin B6, vitamin B12, methionine, and folate, leading to a lower concentration of S-adenosylmethionine in the liver, and thereby cause abnormal DNA methylation[51]. Thus, alcohol can impair the bioavailability of dietary folate as well as folate-dependent intermediary metabolisms to cause carcinogen[52]. Besides, monocyte chemoattractant protein-1 (MCP-1) is a chemokine that plays an important role in regulating tumor microenvironment and metastasis[53]. Alcohol can increase the expression of MCP-1 and its receptor CCR2 at both protein and mRNA levels[53]. The study demonstrated that alcohol may promote the metastasis of CRC through modulating GSK3β/β-catenin/MCP-1 pathway[53].

Cigarette: Cigarette smoke is considered as a risk factor for CRC. A study found that individuals with heavy, long-term cigarette smoke exposure were significantly younger at the time of CRC diagnosis compared to lifelong never smokers[54]. And smoking is also correlative to some specific subtypes of CRC such as MSI-high, CpG island methylator phenotype (CIMP) -positive, and BRAF mutation-positive subtypes[55]. This finding from the study also indicated that epigenetic modification may be involved in smoking-related carcinogen[55]. In general, heterocyclic aromatic amines and polycyclic aromatic hydrocarbons may play an important role in colorectal cancer associated with smoking[56,57]. N-Acetyltransferases 1 and 2 (NAT1 and NAT2) is also considered to participate in the metabolism of aromatic and heterocyclic aromatic amines[56]. Glutathione S-transferases (GSTs), particular GSTM1, GSTT1 and GSTP1 are detoxification enzymes that have been known to metabolize a wide range of carcinogens from cigarette smoke, such as heterocyclic aromatic amines and polycyclic aromatic

hydrocarbons[57]. Thus, NAT1 and NAT2, and GSTs gene polymorphisms may be involved in cigarette smoking-CRC risk[56,57]. A study demonstrated that individuals with fast acetylation capacity achieved by NAT1 and NAT2, may more efficiently activate heterocyclic aromatic amines, thereby increasing the induction of DNA damage and, consequently, more susceptible to CRC[56]. Besides, GST gene polymorphisms influence interindividual susceptibility to smoking-associated CRC,which can play an important role in the in the detoxification of colorectal carcinogens during smoking[57]. A novel opinion is that smoking may increase cancer cell survival and induce some events associated to epithelial-to-mesenchymal transition (EMT) process[58]. Smoking may reduce cell necrosis, deregulate Claudin-1 and E-cadherin expression and enchance the expression of miR-21 to induce EMT[58].

Aspirin: Abundant evidence indicates that regular use of aspirin is associated with a significant reduction in the incidence of CRC[59-62]. Not only that, the beneficial function of aspirin may be emphasized in some specific molecular subtypes of CRC. Several studies have indicated that regular use of aspirin is associated with better prognosis and clinical outcome in COX-2-positive and PIK3CA-mutated CRC[61,62]. Aspirin might inhibit the expression of COX-2 to reduce the prostaglandin E2 (PGE2) synthesis, and thereby to reduce the inflammatory response and suppress cancer cell proliferation and survival[59,61]. In the status of PIK3CA mutation, PI3K and the downstream Akt pathway can be activated to enhance COX-2 activity and PGE2 synthesis, resulting in inhibition of apoptosis in CRC cells[61]. Therefore, aspirin can attenuate phosphatidylinositol-3 kinase (PI3K) activity through inhibiting PGE2 signaling[59,61,63]. Meanwhile, aspirin might inhibit mTOR, a downstream effector of the PI3K pathway by activation of adenosine monophosphate-activated protein kinase (AMPK) in CRC[61]. In addition, aspirin may also inhibit Wnt signalling either directly or through downregulation of PGE2 to suppress the onset of CRC[63].

Besides, We discuss the interaction of diet and lifestyle and tea polyphenols on colorectal cancer.The modification is made as following:

Diet and lifestyle

The impact of diet and lifestyle should be considered in elucidating the relationship of between TPs and CRC risk. In a dietary pattern, tea should be combined with other foods. The combination may enhance or diminish the relationship of tea and CRC. Nowadays, existing studies have proved the relation of tea and some foods on CRC. A study suggested that green tea and black tea can inhibit the formation of heterocyclic amine produced from red and processed meat to suppress colonic aberrant crypt foci (ACF) in the rat[185]. Besides, tea can also induce cytochromes P450 and Phase II enzymes in a manner consistent with the rapid metabolism and excretion of heterocyclic amines[185]. Meanwhile, tea has been shown to block N-nitroso compounds formation from red meat, and thereby exert the protective effect of CRC[186]. A study proved that combination of Se and green tea is more effective in suppressing CRC is than either agent alone[187]. The preventive effect of combination diet on CRC is evidenced by restoring SFRP5 gene expression, increasing histone H3 acetylation and reducing DNA methyltransferase (DNMT) 1 expression, inhibiting β-catenin nuclear accumulation, reducing cyclin D1 expression and cell proliferation in normal-appearing crypts[187]. Other foods should also

been considered when discussing the protective effect of tea on CRC. Further studies should be undergone to understand the relationship and mechanism profoundly. Variations in lifestyle factors might cause the inconsistent findings regarding green tea intake and CRC risk in several epidemiological studies. A study has proved that high green tea consumption(≥25.50g/day) was associated with a decreased risk of CRC, with or without considering lifestyle factors[188]. However, moderate green tea consumption increased the risk of CRC among ever-smokers, ever-drinkers and the high-inflammatory diet group [188]. Similarly, another study in Shanghai also demonstrated that regular consumption of green tea may reduce colorectal cancer risk among non-smokers, but no significant association was found among smokers[189]. However, we can not obtain the relative studies to understand the interaction of PA or aspirin, and tea, on CRC. Furthermore, the relative mechanism of this interaction of between lifestyle and CRC should also be investigated in the future.

2) **This paper discuss the microbiome, but do not adequately discuss the immune system. The authors should discuss it.**

Response: Thank you for the precious suggestion.According to this review,we reviesed our manicript from two aspects.On the one hand,we discuss the effect of gut microbiota on immune system.The modification is made as following:

Gut microbiota can regulate some immune cells of immune system to impact the development of CRC. **T lymphocytes:** Gut microbiota can exert the important effect in T lymphocytes to modulate the progression of CRC. On the one hand, gut microbiota plays an important role in triggering chemokines production such as CCL3, CCL4, CCL5, CCL20 and CXCL10 ultimately leading to T cell recruitment in tumour tissues and improved prognosis of CRC[89]. And bacteria-induced chemokine gene expression may be initiated by Toll-like receptor(TLR) triggering on CRC cells[89]. On the other hand, gut microbiota can regulate the differentiation of T lymphocytes. Different T lymphocytes can exert different effects on CRC. Th1 cytokine interferon (IFN) γ plays an anti-tumorigenic role, whereas the Th2/Treg cytokines IL-4, IL-5, and IL-10 mediate a pro-tumorigenic role[90]. Besides,Th17 cells are known to be pro-tumorigenic in CRC and IL-17A is also linked to the gut microbiota[90]. Gut microbiota depletion can increase numbers of anti-tumor IFNγ-secreting T cells and decrease numbers of pro-tumor IL-17A and IL-10 secreting immune populations to reduce the development of CRC[90]. Another study also demonstrated that a remodel gut microbiota can enhance anti-inflammatory capacity through promoting the induction of Tregs[91]. In addition, F. nucleatum, a proinflammatory bacterial species in tumor tissue but rarely found in normal intestinal microbiota, is associated with increased lymph node metastases and a worse outcome in CRC patients[92,93]. F. nucleatum is likely to possess immunosuppressive activities by inhibiting human T-cell responses[94,95]. F.nucleatum has been shown to expand myeloid-derived immune cells, which inhibit T-cell proliferation and induce T-cell apoptosis in colorectal cancer[95,96]. F. nucleatum expresses the virulence factor FadA on the bacterial cell surface, which has been shown to activate the Wnt signaling pathway and downregulate the T cell-mediated antitumor immune response[92]. Similarly, F.

nucleatum can recruit proinflammatory cytokines, such as IL17A, TNF, and CCL20, which induce inflammation and suppress immunity[97]. Meanwhile, rats with depletion of gut microbiota also showed an increase in CTL cells[98]. A study demonstrated that fecal bacteria from CRC patients can upregulate degranulation and cytotoxicity of CD8+T cells[99]. **B lymphocytes:** The human gut homeostasis requires microbiota coated by both Secretory immunoglobulin M (SIgM) and Secretory immunoglobulin A (SIgA) emerging from B lymphocytes[100]. SIgA deficiency will cause dysbiosis, which may promote the development of CRC[100]. The study indicated that SIgM may emerge from pre-existing memory B cells and could help SIgA anchor highly diverse commensal communities to intestinal mucus[100]. Meanwhile, IL-33 might participate in modulating the IgA-microbiota axis to prevent IL-1α-dependent colitis and tumorigenesis[101]. IL-33 can promote IgA production to maintain gut microbial homoeostasis and inhibit IL-1α-mediated inflammation to prevent the onset of CRC[101]. Similarly, bacteria in CRC can also induce the production of IL17, which promotes influx of intratumor B cells that promote tumor growth and progression[102]. **Natural Killer(NK) cells:** Some certain bacteria may favour recruitment of immune cells such as Natural Killer(NK) cells other than T cells, to achieve favour prognosis[89]. NK cells and CD8+T cells crosstalk in the tumor microenvironment may benefit patient outcome[103]. Nlrp3 inflammasome components had exacerbated liver colorectal cancer metastatic growth by impairing IL-18 signaling and further impacting maturation of hepatic NK cells[104]. In addition, Nlrp3 activation might be mediated by a microbial ligand derived from the remaining intestinal microbiota[104]. **Neutrophils:** Neutrophils are also believed to modulate growth of colon tumors, and correlate with outcomes of patients with colon cancer[102]. It has been indicated that neutrophil depletion is correlation with increased numbers of bacteria in tumors and proliferation of tumor cells, and an inflammatory response mediated by IL-17, and thereby inducing the development of CRC[102]. **Eosinophils:** Eosinophils in CRC patients is strongly linked with a decreased disease risk, better prognosis and extended patient survival[105]. Dysbiosis might impair eosinophil-driven responses to promote the development of CRC[105]. However, the specific mechanism is not clear. **Macrophages:** Macrophages are also involved in the development of CRC. Monocytes/macrophages may polarize as M1 or M2 cells[106]. Overall, M1 macrophage display a pro-inflammatory potential mediating antitumor activities while M2 macrophage display an anti-inflammatory promoting cancer cell growth[106,107]. In tumor microenvironment, tumor-associated macrophages(TAMs) undergo polarization into M1 and M2 phenotypes[108]. The specific interaction of gut microbiota and macrophges on CRC is still required to investigate further. Some studies have provided the insights of the relative mechanism. A metastasis-related secretory protein cathepsin K (CTSK), activated by the imbalance of intestinal microbiota, stimulates CRC progression through accelerating M2 polarization of TAMs in a TLR4-mTOR-dependent pathway[108]. Besides, another study also indicated that defects in the subepithelial band of lamina propria-indigenous macrophages (SBLP-M) barrier in IBD encourage the trespassing of the gut microflora into the host, thereby destabilizing host immunity and promote the development of CRC[109]. High amounts of F. nucleatum intratumoral are correlated with increased macrophage infiltration and CDKN2A promoter methylation in MSI-H CRC[110].

Although it can be hypothesized that the repression of CDKN2A via promoter methylation may be connected with the increased M2 macrophages in F. nucleatum-high CRC, the M2 macrophage density was not significantly associated with F.nucleatum status in MSI-H CRCs, displayed by the study[110]. Besides, a strong association between lower frequency of macrophages, increased Firmicutes, and decreased tumorigenesis was also observed in CRC[111]. **Dendritic cells(DCs)**: DCs play critical roles in maintaining tolerance and immune homeostasis in the gut[112]. And some species in the gut can also induce DCs maturation and the induction of Tregs and IL-10 production to regulate tumorgenesis[112]. Overall, specific immune-microbiota mechanism need to be proved by more animal studies and epidemiological studies.

On the other hand, we also discuss the effect of gut microbiota on immune system when tea polyphenols exert the effect on colorectal cancer.The modification is made as following:

TPs might regulate the immune system through modulating the gut microbiota, in the tumor microenvironment. It has been hypothesized that TPs may promoting T lymphocytes proliferation and reduce M2 macrophages. Besides, other immune cells such as B lymphocytes, Nk cells, neutrophils, eosinophils and dendritic cells may be regulated to some extent respectively. However, there is not enough evidence to confirm these speculations. Further research on specific mechanisms of TPs regulating immune cells through gut microbiota should be undertake. Not only that, different form of TP might exert different impact on lymphocytes. In bulk form of TPs produced a statistically significant reduction in DNA damage in the lymphocytes[268]. In contrast, in NP form of TPs, although initially causing a reduction,produced a statistically significant increase in DNA damage in the lymphocytes[267]. This finding may support TPs can act as both anti-oxidant and pro-oxidant. This study also suggests whether the diifferent form of TPs can play different roles in other immune cells uch as macrophage,granulocyte and different subtype of lymphocytes.More investigations are also required to be carried out in the future.

3) In this context, the authors should discuss molecular pathological epidemiology (MPE), which can investigate environmental and lifestyle factors and molecular pathologies. MPE deeply studies effects of external and internal factors on phenotypes of disease outcome such as cancer, using molecular pathologic analyses of human specimens. Related to this point, the authors should discuss strengths and pitfalls of MPE, which have been described in Epidemiol 2016 and Annu Rev Pathol 2019.

Response: Thank you for the excellent suggestion.It really provides us for a new direction of research, and it helps us establish the novel and accuracy causal mechanism of colorectal cancer.After reading the literatures including Epidemiol 2016 and Annu Rev Pathol 2019 and so one, We think that we should not only discuss MPE on the field of colorectal cancer,but also apply MPE research to study the preventive and therapeutic effects of tea polyphenols on the different subtypes of colorectal cancer.Thus,we revised our manuscript as following:

First,we introduced the MPE and the application on colorectal cancer.

Molecular pathological epidemiology (MPE): MPE is emerged as an integration of molecular pathology and epidemiology, to address the need to investigate the inherent

heterogeneity of pathogenic processes even for a single disease entity[113,114]. Overall, MPE discusses the interrelationship between exogenous and endogenous factors, tumoral molecular signatures and tumor progression[114]. On the one hand, MPE can uncover potential risk factors that are not detectable in conventional epidemiological research without using molecular pathology methods[115]. On the other hand, MPE can help us refine the association between exogenous or endogenous factors and validate specific etiological hypotheses, thereby augmenting causal inference[113-116]. Meanwhile, MPE study can provide novel etiologic and pathogenic insights, potentially contributing to precision medicine for personalized prevention and treatment[116,117]. In addition, MPE can also integrate several disciplines to evolve subfields of MPE including pharmaco-MPE, immuno-MPE, and microbial MPE, to provide novel opinions into underlying etiologic mechanisms[116]. Some progression has been made in CRC. The MPE research has determined the strength of the association for between the exposures and the specific subtypes of CRC, which can help to establish causality and speculating the relative mechanism of exposure acting on CRC. A MPE study has demostrated that both obesity and physical inactivity are associated with a higher risk of CTNNB1 (β-catenin)-negative colorectal cancer but not with CTNNB1-positive cancer risk[118]. Hence, the study implied that energy balance and metabolism status might exert the impact on the development of CRC independent on WNT/β-catenin activation[118]. And then, pharmaco-MPE, integrating MPE into pharmacoepidemiology, plays a vital role in identifying target individuals who most likely benefit from use of a particular drug clinically[116]. MPE studies demonstrated that regular use of aspirin can reduce the risk of CRC with overexpression of COX-2 but not with weak or absent expression of COX-2[119,120]. Many pharmaco-MPE studies has proved that regular aspirin use was associated with lower risk of BRAF-wildtype and PIK3CA-mutated CRC but not with BRAF-mutated and PIK3CA-wildtype CRC[121,122]. Immuno-MPE, the integration of immunology and MPE, can mainly discuss exposures impacting CRC through regulating the immune system and disease-immune interactions[117]. A MPE research has demonstrated that the association of aspirin use with CRC survival is stronger in patients with the programmed cell death ligand 1 (PD-L1)-low tumors than PD-L1-high CRC[123]. It indicated that PD-L1 expression might serve as a biomarker that predicts resistance to aspirin use[123]. In addition, microbial MPE is also studied in CRC. Typically, high level of F. nucleatum might be associated with molecular features of CRC,including MSI-high and CIMP-high[124,125]. Meanwhile, another MPE study demonstrated that a greater amount of F.nucleatum was associated with a lower density of CD3+T cells in CRC, indicating that the interaction of target microbiota and immune system should be discussed further for CRC prevention and precision treatment[126]. In addition to F.nucleatum, other components of gut microbiota need to be investigated in the future. Although the MPE has many strengths, the pitfalls and challenges should be considered. Challenges in MPE mainly include sample size selection, need for rigorous validation of molecular assays and study findings, and paucities of interdisciplinary experts, education programs, international forums, and standardized guidelines[113]. In addition, MPE research need to face the issue of multiple hypothesis testing, so it is necesseary to form a priori hypotheses based on earlier exploratory findings or on potential biological

mechanisms[114]. Similarly, MPE also may create a higher chance to yield spurious findings[113].

And then,we think MPE should be applied when discussing the effect of tea polyphenols on colorectal cancer.Thus, we have added the following:

MPE

Nowadays, there is still lack of MPE studies of TPs on prevention and treatment CRC. However, actually, MPE studies on TPs and CRC are worthy carrying out. These studies can uncover many aspects, not only different kinds of TPs, but also different subtypes of CRC. In addition, whether other endogenous or exogenous factors will impact the effect of TPs on CRC still exist undoubts, And MPE studies may deal with this issue. Moreover, the pharmaco-MPE, immuno-MPE, and microbial MPE should be considered in the impact of TPs on CRC. These are novel and important directions for investigators in the future. MPE studies are beneficial to understand potential causal mechanism of TPs acting on CRC. MPE studies are also helpful to diagnosis and treat CRC using TPs earlier and more precise in clinic. Overall, MPE studies might be a essential direction studying the TPs and CRC in the future to satisfy the requirement of precision medicine.