Format for ANSWERING REVIEWERS

May 15, 2016

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 25750-review.doc).

Title: New Trends in Molecular Biomarker Discovery for Colorectal Cancer

Author: Parisa Aghagolzadeh, Ramin Radpour

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 25750

The manuscript has been improved according to the suggestions of reviewers:

1 Format has been updated

2 Revision has been made according to the suggestions of the reviewer

A mainly well-written, comprehensive review paper.

Authors: Thanks for your positive comment.

Comments;

1. Authors should include septins and their role

Authors: the following paragraph is added to the "Aberrant DNA hypermethylation in CRC

" section:

The *SEPT9* gene methylation assay has recently been developed as a novel blood-based test for CRC (Epi proColon 2.0, Epigenomics AG, Germany).

Septins are a group of conserved GTP-binding proteins which are scaffolding proteins and provide structural support during cell division and compartmentalization [59]. In human, there are 13 known septin genes (*SEPT1* to *SEPT13*). All septins can form heteromeric complexes; within these protein family, *SEPT9* occupies a terminal position in an octameric septin complex and plays a key role in subunit polymerization and the whole octamer stabilization [60]. Therefore, abnormal *SEPT9* or no *SEPT9* may seriously affect cytokinesis. The crucial role of *SEPT9* in the septin complex may be a key factor in CRC carcinogenesis when the promoter region of the *SEPT9* gene is aberrantly hypermethylated and the transcription is compromised [61]. To date, more than 10 independent clinical trials have proved the aberrant *SEPT9* gene methylation as a specific biomarker for CRC early detection and screening.

2. Also include fecal DNA testing as possible new options in the armamentarium

Authors: The "Stool biomarkers" section was restructured and expanded as following:

The presence of tumor markers in stool can be attributed to leakage, exfoliation, or secretion [142]. Exfoliated and secreted markers come from vital and apoptotic colonocytes, shed into the colorectal lumen. Since the stool markers are directly derived from the tumor cells, assumed to be highly specific biomarkers for CRC. These stool biomarkers include stool DNA (sDNA) which can be used for checking the MSI, DNA methylation or mutation for specific cancer related genes, microRNAs, protein biomarkers as well as secretory molecules and biochemical materials resulted from metabolism of cancer cell. Considering the user-friendly features of noninvasiveness and off-site testing along with the potential for low program costs, stool biomarkers warrant reappraisal. Recent technical advances have led to the next generation of stool tests that promise substantially higher

detection rates for the CRC. These biomarkers should be very sensitive with higher specificity because positive test results lead to unnecessary, potentially morbid, and costly colonoscopies [143].

Human DNA is less than 0.01% of the total stool DNA and the vast majority (99.99%) of sDNA is derived from intestine bacterial or dietary; therefore, one of the important technical challenge of sDNA testing is specific detection of methylated or mutated human DNA within a pool of nontarget DNA [144, 145]. Within the past few years, several groups have reported clinical results using different nonoptimized sDNA tests. Several panels of methylated genes within sDNA have been reported for different stages of CRC, involving *BMP3, CDH1*, *CDH13*, *CRBP1*, *CXCL12*, *ESR1*, *HLTF*, *ID4*, *IRF8*, *ITGA4*, *MINT1*, *MINT31*, *NDRG4, P14*, *P16*, *RUNX3*, *SFRP1*, *SFRP2*, *SLC5A8*, and *TIMP3* [146]. These panels were differing in the marker selection, assay methods, and patient populations studied which many of them could not be further validated in the bigger cohorts.

Although several stool based tests (fecal immunochemical test (FIT), guaiac fecal occult blood test (gFOBT), immunological fecal occult blood test (iFOBT), and sDNA test) are clinically available for CRC detection, the specificity and sensitivity for individual tests is not sufficient due to many factors such as inconvenience in sampling or misinterpretation. Recently, the US food and drug administration (FDA) has approved the Cologuard® test (Exact Sciences Corporation, United States). Cologuard® is a panel of multi-target sDNA test, which combines molecular tests for *KRAS* and *β*-catenin mutation, aberrantly methylated *BMP3* and *NDRG4* gene promoters and human hemoglobin immunochemical assay [147].

Good Review

Authors: Thanks for your positive comment.