ANSWERING REVIEWERS

April 08, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format.

Title: Gene Expression Profiles of Peripheral Blood in Colorectal Cancer

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Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 8662

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

3 References and typesetting were corrected

Manuscript Review Result for Revision
Reviewed by 02854680

Manuscript Number	8662
Manuscript Title	Gene Expression Profiles of Peripheral Blood in Colorectal Cancer
Review Time	2014-01-25 00:23

Comments To
Authors
This paper is very well written and examines the possibility of using a panel of genes as a potential biomarker of CRC. Peripheral leucocyte gene expression was quantified using PCR.

- We thank for the Reviewer encourages.

The authors used a pooled multivariate analysis to select genes of interest from a list of CRC candidate genes. The authors then compared their own panel of genes from peripheral blood, to microarray data sets from colonic tissue (CRC and control).

Major limitations.
– Was a microarray used to identify the initial list of CRC genes of interest from peripheral blood? Or were these candidate genes described in previous studies, and were these genes differentially expressed in blood or colonic tissue.

- We thank for the Reviewer pointed out an important issue. We have added and revised the texts in the manuscript as well as the following,

In the Introduction:

…. 28 cancer-associated candidate genes from Quyun, et al. [1] in the peripheral blood samples from 111 colorectal cancer patients and 227 non-cancer controls ….

In the Materials and Methods:

…. Furthermore, as table 1, we validated the 17 CRC-associated genes from Quyun, et al. (Model 1: 5 genes), Marshall et. al. (Model 2: 7 genes) and Han et. al. (Model 3: 5 genes) ….

- Surely a better study design would be to perform a meta-analysis of public microarray datasets, find a the best performing panel of genes, and validate these genes in peripheral blood

- We thank for the Reviewer encourages.

-Were statistics corrected for multiple testing?

- We thank for the Reviewer pointed out an important issue. We have added and revised the texts in the manuscript as well as the following,

In the Materials and Methods:

The Bonferroni adjustment for multiple testing was performed using SISA[1] to control for a family-wise error rate of 0.05, which significant level is considered as 0.05/42 = 0.00114. The p-values in the tables are reported in scientific notation if too many digits were needed for evaluation and to address the issue of multiple testing.

-For what reason should gene expression from peripheral blood leucocytes be similar to gene expression from colonic tissue?(i.e. microarray datasets)

- We thank for the Reviewer pointed out an important issue. We have added and revised the texts in the manuscript as well as the following,

In Discussions:

Circulating cancer cells from any cancer type are capable of disseminating from solid tumor tissues, penetrating and invading blood vessels and circulating in the peripheral blood [2-3]. The number of circulating tumor cells has been used to predict the clinical outcome of cancer patients [4-5]. On the basis of the presence of circulating tumor cells, we identified five molecular markers, MDM2, DUSP6, CPEB4, MMD, and EIF2S3, which were differentially expressed between peripheral blood samples of CRC patients and healthy controls.….

…. Both mRNAs and proteins in the peripheral blood have been tested for diagnostic use to detect circulating tumor cells of different solid tumors or to determine prognoses of various cancers…..

- It appears the accuracy of the 5 gene model is largely driven by the results of one gene (DUSP6), what happens to

results when this gene is removed from analyses Overall it is a laudable concept to develop biomarkers from peripheral blood as this would be useful for national screening programmes, and a PCR assay for a panel of 5 or so genes would be a feasible methodology for widespread utilisation. If the authors (as may be interpreted from the introduction) suggest that changes evident in peripheral blood arise from tumour cells metastasising prior to presentation, this would invalidate the method as a screening method, as patients with metastatic disease would be picked up, and would not be useful for early detection and prevention of metastasis. May be worthy of touching on this point in the discussion.

- We thank for the Reviewer encourages us and pointed out an important issue. We have added an analysis of logistic regression with crude statistics of individual candidate gene and revised the texts in the manuscript as well as the following,

In Discussions, we added ones for the future works:

For the future works:

In the future works, the expression signature of these CRC-associated genes should be evaluated for early detection of CRC, with more samples randomly screened from the population; in addition, subjects who eventually receive a diagnosis of CRC should be evaluated as well. Early CRC detection could provide inherent benefits to the patient and could also enable screening for post-operative residual tumor cells and occult metastases, an early indicator of tumor recurrence. Early detection could thus improve survival in patients before symptoms are detectable, during treatment, or during remission.

Additional analysis:

It appears the accuracy of the 5 gene model is largely driven by the results of one gene (DUSP6), the results when this gene is removed from analyses as the following figure. The crude accuracy of individual candidate gene of *MDM2*, *DUSP6*, *CPEB4*, *MMD*, and *EIF2S3* is 0.735, 0.747, 0.765, 0.722, and 0.660, respectively.

	Crude						
	OR	**95% CI**	H-L	R2	Accuracy	Specificity	Sensitivity
MDM2	5.353	(2.568-11.160)	0.137	0.204	0.735	0.803	0.700
DUSP6	5.065	(2.857-8.981)	0.610	0.364	0.747	0.766	0.684
CPEB4	4.238	(2.542-7.064)	0.441	0.327	0.765	0.800	0.681
MMD	1.592	(1.385-2.750)	0.032	0.138	0.722	0.728	0.692
EIF2S3	0.586	(0.322-1.065)	0.653	0.027	0.660	0.663	0.500

Classification
Grade C (Good)

Language evaluation

Grade B: minor language polishing

Conclusion

Accept

- We thank for the Reviewer encourages and points out an important issue

Sincerely yours,

Chi-Ming Chu

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3 Carmeliet P, Jain RK. Angiogenesis in cancer and other diseases. *Nature* 2000; **407**(6801): 249-257 [PMID: 11001068 DOI: 10.1038/35025220]

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