Authors' response

We sincerely appreciate the interest in our recent article published in *Gut*[1] and the comments raised. The comments by Heneghan *et al* raised some important questions concerning the emerging circulating microRNA (miRNA) aspects of cancer diagnostics. These comments include: (1) the choice of circulating medium; (2) the choice of endogenous control; (3) premature for colorectal cancer (CRC) screening; and (4) whether elevated miRNAs in plasma reflect a general cancer phenomenon, or a true CRC occurrence.

In response to comment 1, based on our experience and commercial kit recommendation, total RNA <50 ng is recommended for quantitative PCR (qPCR) of miRNA. A large amount of RNA cannot improve qPCR results and so is unnecessary. Although we agree with the authors that total RNA extracted from whole blood generates a higher yield than that from plasma or serum because a high percentage of RNA/miRNA is derived from the cellular portion in whole blood, one concern about using whole blood for cancer diagnosis is whether the elevated miRNAs identified are primarily derived from the tumour itself or are simply a secondary response of blood cells during tumourigenesis. If the elevated miRNAs are mainly due to the response of blood cells, those miRNAs may not reflect the patient's cancer phenomenon and so lower the testing accuracy. Heneghan *et al* recently showed that miR-195 and let-7a are elevated in blood from patients with breast cancer. However, a previous study by the same group of authors demonstrated that let-7a is suitable as an endogenous control for qPCR in breast cancer.[2] So, this raises the issue that let-7a elevation in blood is probably due to a secondary phenomenon such as inflammation from blood cells. Accordingly, using whole blood for this diagnostic purpose is questionable.

In response to comment 2, ideally an absolute quantitation approach with standard curve calibration is recommended to be used for qPCR in the field of diagnostics. For relative quantitation, there is still no consensus on the use of an internal normalisation control in plasma. Downregulation of miR-16 has been reported in several cancers including leukaemia, pituitary adenomas, prostate carcinoma and lung cancer.[3–5] In our laboratory, we also found that miR-16 in plasma was aberrantly expressed in patients with breast cancer (unpublished data). Thus, the use of miR-16 as an internal normalisation control in whole blood is still questionable. Furthermore, it was surprising that the same group of authors previously recommended let-7a as one reliable endogenous control in breast cancer.[2] Accordingly, let-7a is not likely to be breast cancer specific and so it raises the issue as to whether let-7a should be used as an endogenous control or diagnostic marker for breast cancer. Thus, an internal normalisation control is still a critical issue for debate. From our point of view, we should eventually switch to an absolute quantitation approach to eliminate the use of an endogenous control.

With regard to comment 3, we agree with the authors that it is premature to apply plasma miR-92 for CRC screening. Larger scale validations are underway, as mentioned in the Discussion section of our original paper.

In response to comment 4, in our paper we showed that elevation of plasma miR-92 and miR-17-3p levels is likely to be derived from CRC. First, miR-92 and miR-17-3p had been selected for further marker validation because of their elevated levels in both plasma and corresponding tumour of patients with CRC. Secondly, their plasma levels were significantly reduced after surgical removal of the tumours. Thirdly, elevation of these miRNAs in plasma due to inflammation, such as inflammatory bowel disease, has been ruled out. Finally, our recent data showed that plasma levels did not increase in other cancer types including breast and gastric cancer. Collectively, miR-92 and miR-17-3p are very likely to be CRC specific.

Enders K O Ng,[1] Jun Yu,[2] Ava Kwong,[1] Joseph J Y Sung[2]

[1]Department of Surgery, The University of Hong Kong, Hong Kong SAR, China; [2]Institute of Digestive Disease and the Department of Medicine and Therapeutics, Li Ka Shing Institute of Health Sciences, The Chinese University of Hong Kong, Hong Kong SAR, China

Correspondence to Professor Joseph J Y Sung, Institute of Digestive Disease, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, New Territories, Hong Kong SAR, China; joesung@cuhk.edu.hk

Competing interests None.

Provenance and peer review Not commissioned; not externally peer reviewed.

Gut 2010;■:1. doi:10.1136/gut.2010.208082

REFERENCES

1. **Ng EKO,** Chong WW, Jin H, *et al*. Differential expression of microRNAs in plasma of patients with colorectal cancer: a potential marker for colorectal cancer screening. *Gut* 2009;**58**:1375–81.
2. **Davoren PA,** McNeill RE, Lowery AJ, *et al*. Identification of suitable endogenous control genes for microRNA gene expression analysis in human breast cancer. *BMC Mol Biol* 2008;**9**:76.
3. **Calin GA,** Cimmino A, Fabbri M, *et al*. MiR-15a and miR-16-1 cluster functions in human leukemia. *Proc Natl Acad Sci USA* 2008;**105**:5166–71.
4. **Kaddar T,** Chien WW, Bertrand Y, *et al*. Prognostic value of miR-16 expression in childhood acute lymphoblastic leukemia relationships to normal and malignant lymphocyte proliferation. *Leuk Res* 2009;**33**:1217–23.
5. **Bandi N,** Zbinden S, Gugger M, *et al*. miR-15a and miR-16 are implicated in cell cycle regulation in a Rb-dependent manner and are frequently deleted or down-regulated in non-small cell lung cancer. *Cancer Res* 2009;**69**:5553–9.