Author’s response to reviews

Title: Mass spectrometry protein expression profiles in colorectal cancer tissue associated with clinico-pathological features of disease

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Sabina Alam PhD
Senior Scientific Editor
BMC series Journals

Dear Dr Alam

Thank you for forwarding the reviewers’ reports on ‘version 2’ of the above manuscript. Referee #2 has asked us to address an issue in a further revision of the manuscript.

Details of our responses to this referee’s comment and the revisions made in ‘version 3’ of our manuscript are summarised below. Changes to the wording in the revised manuscript have been highlighted by underlining relevant sections of the text.

We are grateful to the referee for her expert critique.

Thank you for dealing with our manuscript

Yours sincerely

Professor John D Norton

RESPONSE TO REFEREE 2’s COMMENT
Identification of potential markers: The referee did raise this issue in her previous report and intimated that we had not (even) addressed the issue in the Discussion. In revising the manuscript, we therefore added a sentence indicating the desirability of identifying peaks that are most useful in discriminating clinico-pathological features (p15 in version 2). Also, in our response to this comment, we pointed out that such an exercise would not really add anything substantial to our investigation whose main aim is to determine whether MALDI-TOF spectral profiling has any potential value in CRC.

In pursuing this issue further, the referee now asserts that this (identification of protein markers) is a well accepted requirement and a mandatory step towards validation. The referee also cites an earlier publication describing SELD-TOF MS profiling in CRC (PMID: 16327996) that we did not refer to in our manuscript.

On the first point, we would question the premise that protein identification is a well accepted requirement in SELDI-TOF and MALDI-TOF studies of this kind. While it is true that some studies have identified one or sometimes more differentially expressed peaks, many have not. Indeed, there is no rational (scientific) reason why mere identification should add any weight to such a study. That the peaks generated in these MS profiles do represent proteins/peptides (that have an identity) is beyond dispute. Even if we had determined the identity of one or possibly more of the differentially expressed peaks in our study, in the absence of a more extensive validation exercise (see below), it is unlikely that the readership be more persuaded of the main conclusions of our investigation.

That said, we agree with the referee that identification of candidate protein markers is a mandatory requirement in validation. However, it was not the purpose of our study to validate candidate protein markers. After initial identification, such an exercise would require an extensive study involving, amongst other things, an immunohistological screen of patient specimens to correlate expression levels with those determined by MALDI-TOF. The purpose of our study was to evaluate the potential value of MALDI-TOF profiling to classify various clinico-pathological features in CRC. The results showed that this approach does indeed have potential value in studies aimed at improved molecular classification of this disease.

As a ‘concession’ to this issue, we have expanded a sentence in the Discussion section of the revised, version 3 manuscript (p15) to highlight the importance of identification of protein peaks in validating the candidature of discriminating protein markers.

Regarding the earlier publication (PMID: 16327996 - Melle et al “Different expression of calgizzarin (S100A11) in normal colonic epithelium, adenoma and colorectal carcinoma”, Int J Oncol. 28: 195-200), the SELDI-TOF data presented in this study was very limited and of a preliminary nature with essentially no analysis (correlation with tissue type); the investigation was focussed on identification of S100A11, isolated by 2-D gel electrophoresis. However, in further revising our manuscript we have now referred to this study (p5, new reference 21); the numbering of other references has been amended accordingly.