Reviewer's report

Title: Metastatic susceptibility locus, an 8p hot-spot for tumour progression disrupted in colorectal liver metastases: 13 candidate genes examined at the DNA, mRNA and protein level.

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Reviewer: Cameron Johnstone

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Summary: This study evaluated chromosome 8p using microsatellite PCR LOH analysis in DNA extracted from colon cancer liver metastases and matched normal blood (n=48). For 11 of the metastases, matched primary tumor was available, and for 6 patients, normal colon, primary tumor, and liver metastasis were all available. The study addresses important issues in colon cancer research from both a basic science and clinical management perspective. Indeed, chromosome 8p has been studied in great detail and multiple different regions implicated in colorectal, prostate, and breast cancer but still no single tumor suppressor gene has been shown. Therefore, haploinsufficiency may be playing a role on this chromosomal arm, and elucidating the relevant genes has been difficult.

The authors have done a great deal of work here and are to be congratulated for forming a large tumor bank of colon cancer metastases (n=48), as access to these specimens is often not that easy and can be an incredible resource for making new discoveries. The manuscript is relatively straightforward in its approach; identify a manageable minimal region of LOH, then perform molecular analyses on candidate genes from that interval. Therefore, the most critical aspect is the initial definition of the minimal region of loss, because all subsequent experiments flow from this, and also because this chromosomal arm has been studied in tremendous detail previously and many conflicting data with respect to the real critical region(s) still exist.

The authors start off by comparing 35 microsatellite markers in metastases and normal blood, and results in the definition of a ~2Mb minimal region of deletion. One danger of this approach is that you simply discover alterations that were present in the primary, i.e. you are not enriching for any changes that are specific to metastases. More information is needed regarding the PCR LOH analyses, especially since this approach has now been superseded by SNP arrays. In addition, the generally very good correlation between mRNA expression levels between primary tumors and liver metastases (but not between normal colon and primary tumor!) actually argue in favor of these genetic alterations occurring at the level of the primary tumor rather than being specific for the distant lesion. Following the qPCR analyses, ADAMDEC1 was clearly the best candidate for further study, though was ultimately disappointing since unfortunately the mRNA and protein levels did not correlate between primary and metastasis.
The manuscript is generally well written although is lacking in specifics in some places. While appearing to begin strongly, the story seems to fade away in the last 1/3 or so, ending in some negative IHC data (Fig. 6) and a confusing reference to gene-gene interactions (Fig. 7). The discussion and conclusion, at 6 pages in length, is overly speculative and actually circular in argument since on page 19 it is stated that permanent mechanisms of gene expression regulation such as methylation and genetic mutation is not occurring during CRC metastasis. These permanent alterations would include genetic alterations such as LOH and deletion/amplification as well, and Figure 1 shows that MSL (metastatic specific LOH) is occurring in a high proportion of patients.

Major Compulsory Changes:

Figure 1:

1) This is by far the most important part of the paper. Since samples from liver metastasis and primary tumor from the same patient were available in 11 cases, this analysis could have been done in 2 ways. Firstly, as presented in Fig. 1, and secondly, as a 3 way comparison among normal blood, primary tumor, and liver metastasis. If this analysis is also performed, then do the same minimal regions of deletion fall out, or are others more interesting? Since NEFL defines the telomeric boundary of the minimal region, it should not be the most telomeric marker shown in the diagram. Since two additional markers are shown centromeric to D8S1734, then two additional markers should be shown distal to NEFL (and what is NEFL, define)?

2) Additionally, in any LOH analysis, the specimens with small interstitial deletions are the most interesting. Indeed 1 specimen (7th from left), has only the minimal region deleted, and specimen #8 has a slightly larger region. However, there is only 1 microsatellite examined that falls within the minimal region (D8S1181). This is a concern. Are there additional microsatellite markers that can be evaluated in this region? If not then are there SNP genotyping PCR kits from ABI that can be used to confirm? Or, could the two interstitial deletions be confirmed in #7 and #8 by analyzing by SNP array? Finally, the ordering should be changed so that all samples confirming the minimal region should be to the left followed by the others.

3) It is stated that 73% (8 of 11) showed metastatic specific loss (MSL) on page 10 and this aspect is supposedly supported by Figure 1c. Firstly, since MSL is entire focus of the manuscript, it needs much more detail. The data in this panel should be shown in a (expanded) schematic similar to the one presented in Figure 1a. The example shown in Figure 1c is confusing, the left panel looks like loss but is the right panel an example of retention? The marker should be written on the panels.

Minor Essential Changes

1) Figure 2: It is stated in the discussion only that the ~2Mb region of deletion contained 23 protein coding genes but only 13 were selected and shown on the
diagram. This should be stated in the Figure legend along with a better explanation of why 10 were not examined. An additional table (Supplementary data?) could identify the other 10 genes.

2) Figure 3: It isn’t stated in the text that cDNA was used as template and why. Might you miss detecting splice site mutations if cDNA is used as template? A positive control showing a patient with intact DR5 expression in normal, primary, and metastatic samples is definitely needed. Also, in the Western blot, expression of C790T is shown in normal and metastasis, whereas in the IHC it is shown in primary, and metastasis. The same tissues should be shown in Westerns and in IHC if possible. Did the patient with C790T have LOH in their primary tumor and/or metastasis? Finally, if C790T is germline heterozygous, then why is there no evidence of a wild-type band in the normal tissue (LN)?

3) Figure 4a: Standard deviation should be used rather than SEM. 4b: The y-axes actually show the expression ratio rather than fold change. Fold change and ratio are not identical for expression ratios <1.

4) Figure 6: While the IHC is well done, this is essentially negative data and can be deleted or moved to Supplementary data.

5) Figure 7: Since gene-gene interactions are not pursued to any great depth, this Figure is superfluous and can be deleted or moved to Supplementary data.

6) Methods: Were the scorers of the LOH data experienced at this and was it done in a blinded way?

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

I declare that I have no competing interests