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.

Version: 2 Date: 18 April 2008

Reviewer: Cameron Johnstone

Reviewer's report:

My comments are numbered in concordance with the numbering system used by the authors in their response.

1) The authors now show additional microsatellite markers centromeric to NEFL in Fig. 1A. However, it now becomes apparent that metastasis-specific loss is very frequent at any given locus and makes the selection of the interval spanning NEFL to D8S1786 (to study further in LM samples) somewhat arbitrary, given the limited number of samples. The follow-up experiment in the LM samples does not clarify the situation, although the selected region (NEFL to D8S1734) seems reasonable in light of the data presented. It was a shame that the most interesting case (#22) was not informative at the two telomeric markers, as retention here would strengthen the claim that this is region is indeed targeted for LOH. It would help if a box could be drawn around the minimal regions selected in A and B. In D, the marker should be indicated above the gels.

2) OK
3) OK
4) OK

5) See response to #1 and also:
This is fine although it would appear at first glance that D8S1048 is not that bad of a marker since in part A it was informative in 4 of 6 cases and 3 of the informative cases displayed metastasis-specific loss.

6) OK
7) OK
8) OK

9) As predicted, the wild-type DR5 band is not present in the C790T patient. However, assessment of the lower band as a 28kDa truncated DR5 is a stretch. The lower band appears to be almost the same size as a presumably non-specific band in the wild-type lane. The IHC confuses matters as it shows the opposite of the Western blotting results. The IHC shows increased DR5 staining in primary tumour and matched metastasis from the C790T individual,
whereas by Western blotting the 28kDa band in C790T is far weaker than the wild-type band in lane 1 (this is assuming that the lower band is 28kDa DR5). Indeed the Western blot is actually consistent with nonsense-mediated decay of the mutant C790T transcript with deletion of the 2nd allele as supported by the sequencing results.

11) OK
12) OK
13) OK

Other points:
1) In abstract, should read “….mutation, quantitative real-time PCR…”
2) Wherever possible 8p21 should be used instead of simply 8p.
3) In background, page 5, I would delete “The first hit is….. to …..involved in metastasis”. The description is too vague and way too simple to adequately capture current understand of how TSGs are inactivated in human cancer. Most readers will be familiar with the two hit hypothesis.
4) Final sentence page 5 should read “…protein levels associated…”
5) Results page 9. There is no way that analysis of 1 marker on 8q can allow one to claim that “….loss did not extend to the long arm”. This sentence should be deleted or re-worded.
6) Page 14. The text under STC and LOXL2 appears to constitute an oxymoron. To paraphrase, it is claimed that gene disruption has been reported previously, but the current study showed overexpression, which was consistent with the literature !! This passage needs to be re-worded.
7) The 2.5 fold lower expression of DcR1 needs a (data not shown) if the primary data is not presented anywhere in the manuscript.
8) I suggest the term “metastasis-specific loss” rather than “metastatic-specific loss”
9) Figures 4 and 5. The results could be presented in a more compelling way. By using a log2 scale in Fig 4A and 5 and a log10 scale in Figure 4B, the expression differences are minimized, or compressed, compared to using a linear scale on the y-axes. For example, in figure 4A, a delta Ct difference of 1 is equivalent to a 2 fold change. As it stands, it does not appear as though there is much deregulation of AdamDEC1, whereas on a linear scale these differences would be brought out. In addition, the inverted scale (large delta Ct value = lower expression) is unnecessarily confusing and counter intuitive.

Similarly for 4B, the differences between primary tumour and metastasis would be more obvious if linear scales were used.
**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