Author's response to reviews

Title: SnoN expression is differently regulated in microsatellite unstable compared with microsatellite stable colorectal cancers

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Author's response to reviews:

Dear Sir / Madam,

Please find attached our revised manuscript entitled "SnoN expression is differently regulated in microsatellite unstable compared with microsatellite stable colorectal cancers" for resubmission to BMC Cancer. We have extensively revised this version in accordance with the reviewers' suggestions as outlined below, which we believe has significantly improved the manuscript.

This is the first report quantifying expression changes in the SnoN gene in colorectal cancers, and the first time SnoN has been examined in the context of microsatellite instability. Our data show that up-regulation of SnoN is observed commonly in colorectal cancers regardless of microsatellite instability status. Importantly, SnoN was also frequently down-regulated specifically in microsatellite instability high (MSI-H) colon cancers. We have correlated our data with TGFRII mutation in MSI-H tumours and prognostic indicators for all samples. Based on our findings we suggest that MSI-H tumours present an excellent model system in which to study the opposing oncogenic and tumour suppressive activities of SnoN.

Kind regards,

Vicki Whitehall, PhD

Reviewer 1 - Kieran Sheahan

Major Compulsory Revisions:

Nil.

Minor Essential Revisions:

This was an error in Table 1. We apologize for the error and thank the reviewer for highlighting this. We have added the 2 stage D MSI-H tumours to the table. Please note that the original analysis applied only to MSS cases so the P-value stated in the table is not affected.

Discretionary Revisions:

We have adjusted the final paragraph of the discussion to highlight that our comments regarding prognosis refer only to MSS cancers, and have added that larger studies are now necessary to allow correction for tumour stage.
We have added Table 2 to present our TGFRII mutation data in relation to SnoN expression levels.

Reviewer 2 - Alexander Dobrovic

Major Compulsory Revisions:

Nil.

Minor Essential Revisions:

We have altered the title to describe in more detail the findings of the paper.

We have altered the graph as requested so that individual data points are presented, rather than as a box and whisker plot. We have edited the legend for Figure 1 to more fully explain the data.

The highlighted spelling errors have been corrected.

Discretionary Revisions:

We have expanded the discussion to further comment on the observed down-regulation in MSI-H tumours, and have discussed possible mechanisms for this down-regulation.

We have added Table 2 which shows which tumours had mutated TGFRII in the context of SnoN expression. In response to the reviewer's question regarding the nine tumours without increased SnoN expression, five of those tumours had SnoN down-regulated whilst the remaining four remained unchanged.

In response to this reviewer's query regarding homogeneity of the MSI-H tumour subgroup, we performed BRAF V600E mutation analysis. Fourteen of the 18 tumours (78%) were mutated at this position. This frequency is consistent with what has been reported in the literature for series' of sporadic MSI-H tumours. For one of the four tumours without the BRAF V600E, our previous methylation results indicated this tumour nonetheless did display the CpG Island Methylator Phenotype (CIMP) as well as a methylated MLH1 allele, suggesting a possible mutation at another site within the BRAF gene. Methylation data was available for 2 of the 3 remaining BRAF V600E wt tumours, neither of which displayed the CpG Island Methylator Phenotype. One of these patients was an 80 year old male and may have a somatic mutation of MLH1. The other patient is a 59 year old male with no family history of colorectal cancer and likewise may have a somatic mutation of MLH1. With regard to our data, there were no statistically significant observations to suggest the four BRAF wt tumours were skewing the results. SnoN expression was upregulated in one, unchanged in another, and down-regulated in the remaining two. Three of the four cancers had a mutation in the TGFRII A10 repeat. We are therefore confident this cohort adequately represents sporadic MSI-H tumours. Although of potential interest in a larger series, we feel that presenting the BRAF mutation data would add unnecessary confusion to our manuscript.

Reviewer 3 - Carla Oliveira

Major Compulsory Revisions:

1. We agree with this reviewer's suggestion that it would be interesting to examine SnoN expression in a larger series of tumours, and suggest this in the final paragraph of our discussion. However, given the inherent difficulties in collecting fresh surgical tissue for examining mRNA expression, we feel that 52 is a considerable cohort size and is a unique strength of this study. In this cohort of molecularly and clinically well-characterised tumours, we have identified a statistically significant correlation between SnoN up- vs down-regulation relative to pathway of tumour progression (MSI-H vs MSS, P<0.01). It would be impossible for us to increase our sample size and do not believe this would be likely to affect our already significant P-value.
2. We apologize for this error in the manuscript and thank the reviewer for being so diligent as to recognise the error. The correct reverse primer sequence has now been entered, which is definitely the primer we used.

3. There are four known Sno isoforms. Our primers recognise SnoN, SnoI and SnoN2, but do not recognize SnoA. SnoI was identified in 1993 (Pearson-White, Nucleic Acids Research, 21: 4632-4638) and has not been reported since. This isoform appears to be expressed specifically in skeletal muscle. There has likewise been only one study examining SnoN2. This was published by the same author in 1997 and described the identification of the human isoform (Pearson-White and Crittenden, Nucleic Acids Research, 25: 2930-2937). SnoN2 is a smaller splice variant of SnoN and represents only 2% of the total transcript population. Whilst potentially interesting, detailed further investigation of these rare isoforms would be complicated and labour intensive, and we believe is beyond the scope of this study.

4. Table 2 has been added to clarify the TGFRII mutation results in relation to SnoN expression.

5. We have modified the discussion consistent with these comments, on page 8.

6. This is an error which has been corrected as noted in response to reviewer 1. Please note that the origina analysis applied only to MSS cases so the P-value stated in the table is not affected.

7. The discussion has been re-written in accordance with this reviewer’s request. In particular we have expanded our discussion of the different pathways of colorectal tumorigenesis with respect to microsatellite instability.

Minor Essential Revisions:

1. The error in the methods section has been corrected.

2. The error in Table 1 has been corrected.

Discretionary Revisions:

Nil.