Author's response to reviews

Title: Epigenetic regulation of L1CAM in endometrial carcinoma: comparison to cancer-testis (CT-X) antigens

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Author's response to reviews: see over
Editorial changes

*We have now introduced a method section in the abstract.*

*The names of the ethic commitees that have approved our work are given on pages 8 (Zurich) and 9 (Innsbruck).*

Reviewer 1

Typos and presentation errors should be checked. For example, on page #12, the new paragraph Methylation of the L1CAM promoter in EC tumor tissues, begins with the sentence “it is quite known that the methylation... The lower case is a typo but the sentence could be rephrased to read, "It is now well known that..."

.....*this change has been introduced.*

Reviewer 2

General comments:
This manuscript is marred by the authors glossing over the data and omitting to look carefully at the detail of what the data is saying. This occurs repeatedly throughout this short manuscript. It is not appropriate to suggest that all the cell lines are giving identical data when they are not, nor is it appropriate to group all of the CT-X antigens together and suggest that their expression levels are always similarly affected by the deacetylase inhibitor or the demethylating agent when they are not. The REST data is also over interpreted.

...*we now have removed the data on REST. We also have tuned down some of our statements.*

Minor Essential Revisions:
Abstract: The authors need to define many of the abbreviations that have been used in the abstract. Readers of the entire manuscript will be able to see the list of abbreviations but readers of data bases like PUBMED would have great difficulty working out whether they wish to down-load the paper without this information. These comments particularly apply to the following: ECs, DNMT1, and HDAC.

*We have now explained all abbreviations in the abstract.*

Background, page 3: “IHC” should be defined.
Page 4, line 11: The words “and patients” were added to the end of a sentence describing the methylation status of the L1CAM promoter. What cells or tissues were examined from these patients? I presume these were colorectal cancer patients, but this is not made clear. This must be clarified.

The concluding sentence should be rewritten. Aim (ii) is to see if L1CAM and the CT-X genes “bear some similarity”, similarity in what way?? I presume this is methylation status, but this should be made clear.

It would be helpful if a paragraph was included in the Background to better explain why L1CAM and the CT-X antigens are being examined for similarities in their epigenetic regulation. In addition, a few sentences of background on the CT-X antigens chosen (MAGE-A4, MAGE-A3 and NY-SSO-1) would aid in the understanding of the manuscript as currently the reader is left wondering why these antigens.

Materials and Methods, page 8: “CC1” should be defined. What control antibodies were used to check the accuracy of the staining data?

Figure 2 A) the Y-axis of the graphs is given as “relative to DMSO mRNA level” what this means in practice and how it is calculated must be explained in either the methods or in the Figure legend. (This comment also applies to other figures where data has been similarly expressed relative to DMSO mRNA level.)

Page 12, line 18: I don’t believe that beta-catenin is a transcription factor in its own right. Beta-catenin does however, bind transcription factors.

Figure 5B: I presume the different numbers on the X-axis refer to different tissue samples, this should be clarified.
Page 13: The text referring to figure 5B suggest that there is “a tendency for hypermethylation of the L1CAM promoter” in the L1CAM positive staining areas. I believe this is far too strong a statement based in the data presented and it should be rewritten to better reflect the data in Figure 5.

...we do not agree since we have clearly indicated that this tendency was only seen in some but not in other samples.

Page 13: The description of the data presented in Figure 6 should be rewritten. For the Hec1A cells following TSA treatment alone there is virtually no effect for MAGE-A3, a very convincing, pronounced effect for MAGE-4A and a minor effect for NY-ESO-1, which is not what is presented in the text. Moreover, to say that at the mRNA level the three CT-X antigens are uniformly strongly upregulated by 5-AzaC or 5AZaC/TSA in Hec1A cells is incorrect based on the data presented. This may be true for the other cell lines but it is not the case for Hec1A cells. There is no attempt to suggest why there is such a big difference between 5AzaC/TSA treatment and TSA treatment alone in relation to expression levels of MAGE-4A in Hec1A cells; some explanation on this is warranted. The conclusion here should be that the sensitivity of both L1CAM and the CT-X antigens to TSA and to DNA methylation changes varies depending on the cell lines tested and the CT-X antigen examined.

...this has been clarified. We point out on page 14 that HEC1A behave different from the other cell lines as they appear to have a general resistance to 5-AzaC-treatment. This is also apparent in fig. 3A where no decrease in the methylation of the L1CAM promoter was seen. We include the final sentence of the referee.

In addition, the authors also should have dealt better with the differences in the Western blotting data and the mRNA data when describing the effects of knocking down DNMTI – is this Western Blotting data consistent? If it is, then this should have been indicated along with the number of times the assay was performed and some comment on the differences observed should be included.

...western blotting (WB) and RT-PCR are techniques that are not directly comparable. RT-PCR analysis gives relative changes. WB gives absolute changes but the affinity and the quality of the detecting antibody is of paramount importance. The number of experiments performed is now given in the legend of the figure.

Page 14: The inclusion of the REST data really seems to come out of the blue. This transcription factor must be introduced in the Background so that this section is properly incorporated into the manuscript. I would suggest that this section be included under the previous heading, with a rationalisation for performing the work being included as an introductory sentence. I do not believe the description of the data in Figure 7D warrants its own section.

There are probably a number of factors involved in the regulation of L1CAM expression and the epigenetic factors examined in this manuscript are probably
only part of the story. By coming to the conclusion that REST is probably not involved in the regulation of L1CAM expression simply because there were no major changes in the levels of REST mRNA following DNMTI knock down and 5-AzaC treatment is an over interpretation. Rather these data reveal that the epigenetic changes caused by knocking down DNMTI, or by treating with 5-AzaC are not major factors in regulating REST transcription.

Conclusions: this section should be modified according to the changes suggested above, in particular the first sentence of paragraph 2 should be rewritten. Similarly the abstract should also be modified to incorporate the suggested changes.

...we agree with the referee that the REST data should be eliminated from our manuscript. This has been done in the present version of the paper.