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
Author’s response to reviews:

Title:

**Old:** Evidence that L1CAM is not a Cancer/Testis (CT-X) antigen  
**New:** Epigenetic regulation of L1CAM in endometrial carcinoma: comparison to cancer-testis (CT-X) antigens

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**Reviewer 1:**

The manuscript, ‘Evidence that L1CAM is not a Cancer/Testis (CT-X) antigen’ by Schirmer et al reports interesting aspects of the regulation and expression of L1 cell adhesion molecule (L1CAM), in endometrial carcinoma and normal testis. However, since it was never proposed to be a Cancer/Testis Antigen (CTA), it is not clear why the authors set out to investigate this. Even though a rigorous definition for the CTAs is lacking, it is fairly obvious that its mere presence on this chromosome or its ability to respond to DNA hypomethylation does not for a logical basis for asking the question whether L1CAM is Cancer/Testis Antigen (CTA). There are 100s of genes on the X chromosome and many of them could (and probably do) respond to 5 aza-cytidine treatment. Therefore, although the authors provide good evidence to support the fact that there are some similarities between L1CAM and the CT-X genes, emphasizing the fact that it is not a CT-X antigen (since it is not expressed) in the testis does not add to the significance to this work particularly because L1CAM was never claimed as a CTA. Thus, the title needs to be changed to reflect this issue. Perhaps, additional experiments using larger cohorts of endometrial cancer or any other cancer to discern its biomarker/therapeutic potential may serve well to strengthen this manuscript. In lieu of such information, the data reported in negative light do not appear to serve any purpose or answer a specific question.

...we agree that it was never suggested that L1CAM might be a CT-X antigen. Our attempt to create a link between these groups of cancer antigens was indeed far-fetched. In the revised version we have changed the title and speak about a comparison between L1CAM and CT-X. We have also included additional methylation data on the L1CAM promoter in EC tumor sample.

The authors need to review the current literature when dealing with the CTAs. For example, the second sentence in the section on Background (pg #3), “More than 40 CT genes have been identified so far, and they can be generally grouped into those, encoded on the X-chromosome (CT-X antigens) and those not encoded on the X-chromosome (non-XCT antigens) [2]” is an old reference; currently >200 CTAs have been identified and catalogued (see
...we have followed this suggestion. Unfortunately, this website given is no longer accessible. As the focus of the paper towards CT-X was now significantly tuned down we have shortened their discription.

Reviewer 2:

It is known that the expression of the CT-X antigens MAGE-A4, MAGE-A3 and NY-ESO-1 is regulated by methylation. It has also been published that L1CAM expression in colorectal cancer is regulated by methylation. Given this I struggle to see the new information that is provided by this manuscript. The authors should make this clear and refer to newly published existing literature. Moreover, it is well known that performing analyses of methylation patterns in cell lines that have been maintained in culture is fraught with potential problems. Aberrant or different methylation patterns from that in the parental tissue is a common result of maintaining cells in tissue culture. Frequently methylation is increased in the cell lines and as a result the expression of many tissue specific genes modified. Indeed, even in this present study it is clear that the methylation levels associated with the genes under study is not the same in all of the cell lines tested. Hence, the question needs to be asked as to the relevance of relating the results obtained on the methylation patterns of genes and gene expression in endometrial carcinoma cell lines to expression patterns in normal human testis tissue. The authors need to explain their thinking in this regard. Why was this study not conducted using testicular cancer cells and testicular cancer cell lines, a comment on this should also be included in the manuscript. A further point of concern is that given L1CAM protein is not expressed at in normal human testis tissue but is expressed in endometrial tissue this, by definition, means that LICAM cannot be called a CT-X antigen. Interestingly, although testicular expression is the defining characteristic of CT antigens, this criterion has only been met based on mRNA analysis for many CT genes and the presence of protein in testis and/or cancer has only been confirmed for a small subset of CT antigens. However, by definition, CT-X antigens are not found on normal endometrial tissue. Given that, one wonders why was the manuscript written with its present focus? May be a solution would be to find another focus for the regulation of expression data in this manuscript, taking into consideration the point made above relating to previously published work.

.... the only study on L1CAM promoter methylation in cancer was published by Kato et al in 2009. This work has neither been confirmed by other groups nor has it been extended to other tumors. In the present paper we have therefore studied ECs since we have previously shown that the L1CAM gene contains two 2 promoter regions, a fact that was not known at 2009.

The reviewer is right that cell line data may be misleading. In the revision we have now included promoter methylation studies in EC tumor tissues. We also included normal endometrial tissue. The comparision of cell lines to tissues indeed suggest that cell lines may over-estimate the impact of methylation on dynamic changes in the tumor microenvironment. Our results give a more realistic picture in contrast to the findings of Kato et al.

The reviewer suggests to study promoter methylation of L1CAM in normal testis and testicular
cancer. This is an interesting suggestion! Due to the lack of samples this could not be done in the present study. However, one of the co-authors (VT) is now conducting such work.

Suggest include all of the primers in a single Table – this would make the methods easier to read.

..for reasons of space we have not created an additional table showing primer sequences.

Legend for Fig 6E is missing and more explanation is needed in the legend to Fig 6D.

...this has been corrected.

The correct nomenclature for the CT-X antigens used in this study is: MAGE-A4, MAGE-A3 and NY-ESO-1. The figures, figure legends and the text should be changed so that this is consistent throughout.

...this has been corrected in all Figures.

Figure 5: The various shades of grey are not sufficiently different to easily distinguish the different bars in the graphs. I suggest using cross hatching and open bars as well as one shade of grey.

...this has been corrected.

Figure 1: The legend identifying the histograms should be included in the main Figure legend, and “gam” is not a general abbreviation, nor is it defined in the text.

...this has been corrected.