Author's response to reviews

Title: Serological identification of Tektin5 as a cancer/testis antigen and its immunogenicity

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Author's response to reviews: see over
Dear Dr Frattini,

We thank the editor and the reviewer for kindly reviewing our manuscript entitled ‘Serological identification of Tektin5 as a cancer/testis antigen and its immunogenicity’ by Hanafusa T. et al. We carefully read the comments and revised the manuscript accordingly.

Major compulsory revisions.

1. The authors use the whole coding region of the TEKT5 gene to produce recombinant protein for the ELISA assay. A figure should be shown to demonstrate whether there is a high degree of homology between different members of the TEKTIN family – if so then it is possible that the autoantibodies detected may not be specific for TEKT5 but may also cross react with other TEKTIN proteins. In this case the authors must use a region of TEKT5 that is specific for TEKT5 only and use this to make recombinant protein for the ELISA.

We added Figure 3 that shows the sequence alignment of five members of Tektins, and indicated the sequence identities were small (p.13, l.18).

2. The paper would benefit from NY-ESO-1 expression studies being performed in the same tumour tissues to enable a proper comparison to be made between these two antigens in malignant as well as the normal tissues. These results should be of value in further evaluating the potential of TEKT5.

We included the data of NY-ESO-1 expressions of the same set of tumor tissues in Figure 1B (p.12, l.2).

3. Error bars should be provided for the results in Figure 2.

We added error bars in Figure 2.

4. The authors mention that they will now go on to look at a T-cell response to TEKT5. However, this may not be the appropriate next step. Surely this should be the investigation of TEKT5 expression at the protein level? There are antibodies commercially available to human TEKT5. The specificity of these reagents should be checked and then used to study protein expression of TEKT5 in normal and malignant tissues.

Protein expression studies by Western hybridization or immunohistochemistry are underway. We will report those data in the future.
Minor essential revisions

1. **Authors cite that 11 positive clones were identified in screening a normal testicular library with colon cancer serum. However Table 2 shows 10.**

Table 2 shows 11 clones of 10 antigens (genes). We identified 2 clones in OY-CO-3 (DLD), so, the clone number is 11 and antigen (gene) number is 10.

2. **Also do the authors mean WDSUB1 rather than WDSAM1 mentioned?**

WDSAM1 is a synonym of WDSUB1. We modified the description WDSAM1 to WDSUB1 to avoid confusion (p.10, l.19).

3. **The authors state that TEKT5 appears to have a ‘high immunogenic potential’. This statement should be modified or explained in the context of other studies using autoantibodies since only a subset of patients had autoantibodies to TEKT5.**

We evaluated the frequency of 13/101 (13%) is high compared with that of NY-ESO-1 (24/443 (5.4%), unpublished). And there is no antibody response in control. We modified the description to show our point clearly (p.14, l.7).

4. **There is a mixture of roman and Arabic numerals for the Table numbers.**

We changed roman numerals to Arabic (p.12, l.8).

We hope that we have responded adequately to the suggestions of the reviewer. We would like to express our thanks to you and the reviewer for the valuable suggestions, which helped to improve the manuscript.

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