Author's response to reviews

Title: CD26 Expression on T-Anaplastic Large Cell Lymphoma (ALCL) Line Karpas 299 is Associated with Increased Expression of Versican and MT1-MMP and Enhanced Adhesion

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Author's response to reviews: see over
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Dear Editor,

Thank you for your recent communication dated September 16, 2013 regarding our paper entitled “CD26 Expression on T-Anaplastic Large Cell Lymphoma (ALCL) Line Karpas 299 is Associated with Increased Expression of Versican and MT1-MMP and Enhanced Adhesion” (MS: 1574079839101660) by Pamela A Havre, Long H Dang, Kei Ohnuma, Satoshi Iwata, Chikao Morimoto and Nam H Dang. We would also like to express our sincere gratitude to the Editor and the reviewers for their effort in reviewing our paper and for their comments, which truly help to strengthen the paper. At this time, we are submitting our revised paper with our point-by-point replies to the reviewers’ comments. We sincerely hope you find our revised manuscript to be acceptable for publication in BMC Cancer.

Below please find our replies to the reviewers.

Jonathan Duke-Cohan

1. The samples were run again on a 7.5% gel (Figure 2A). The top of the gel is indicated. The parental Karpas 299 cell lane contained a band that remained at the top of the gel. The 250 kD marker is indicated. This result is consistent with others that do not digest with chondroitinase ABC. Although we used digestion in the initial gels, screening clones was simpler if the digestion step was omitted. To confirm the identity of versican, RT-PCR was run using V0 and V1 specific primers (see Figure 2B).

For others who have omitted digestion or run with or without prior digestion, please see Figure 1B and 1C, first lane labeled ‘V1’ in Sheng et al., Molecular Biology of the Cell, 16, 1330-1340, 2005 and Figure 2A in Arslan et al., British Journal of Cancer 96, 1560-1568, 2007 and Figure 4 (lanes without digestion) in Dours-Zimmermann, Journal of Biological Chemistry 269, 32992-32998, 1994.

2. The suggested change to “Microarray analysis revealed that…” was made on page 4.

Thomas Wight

1. The model has been simplified.

2. We have demonstrated that expression of versican (V0/V1) was higher in the parental Karpas 299 cells than in CD26-depleted clones at a ratio of at least 80:1. The parental Karpas 299 cells expressed higher collagenase I activity than the CD26-depleted clones or the versican knock-down clone 6RD3. Since collagenase I degradation is essential for penetration of the extracellular matrix, and MT1-MMP is one of the key enzymes involved in degradation of the extracellular matrix, we evaluated its expression on the cell surface by both surface biotinylation and flow cytometry and found that MT1-MMP was highest in the parental Karpas 299 cells. We
next monitored CD44 status, which is important for the localization of MT1-MMP to the invadopodia, and also found that CD44 secretion/cleavage was highest in the parental Karpas 299 cells. Finally, we evaluated Erk (1/2) activation in vesicles isolated from spent media, and demonstrated that Erk (1/2) activation was also at the highest level in the parental Karpas 299 cells. These findings are the basis for the model shown in Figure 1.

3. We agree with the reviewer that this is a working hypothesis and that future experiments would be needed to confirm the functional relationships among the molecules shown in the model. The model is intended to serve as a guide that will be subjected to modifications and refinements based on future observations.

Sincerely,

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