Reviewer's report

Title: Gene expression profile of AIDS-related Kaposi's sarcoma: role for sialoadhesin/CD169 in tumours and circulating blood cells

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Reviewer: Patrick PS Moore

Level of interest: A paper whose findings are important to those with closely related research interests

Advice on publication: Unable to decide on acceptance or rejection until the authors have responded to the compulsory revisions

This is a novel experimental approach to understanding gene expression in KS tumors and hence has value. SAGE has unique advantages over microarray approaches in that novel and previously unknown transcripts are catalogued. Further, SAGE theoretically provides an absolute standard allowing independent comparisons by other investigators against an experimental expression profile. In this case, two KS skin lesions were compared to a pooled sample of normal skin from breast reduction surgery. Of the differentially expressed genes, five genes were selected for further study by semi-quantitative RT-PCR because of their specificity only to the KS lesions. Surprisingly, despite large differences in SAGE expression, RT-PCR revealed minimal differences that probably reflect limitations in RT-PCR. Despite this, sialoadhesin expression is a potential diagnostic marker that warrants further study. One of the most interesting findings is direct detection of viral transcripts by SAGE. Overall, the study is technically very well performed and meticulous although conclusions are biased for reasons outlined below. The primary importance of this data is in providing a baseline SAGE profile of KS lesions that can be for comparison by other SAGE projects.

Major Comments:

1. Tissue selection: KS is an endothelial cell tumor but is compared here to epithelial tissue; it is not clear that differential gene expression represents a tumor-specific or a tissue-specific process. Since SAGE results allow (in theory) valid in silico comparisons, comparison to existing endothelial cell libraries deposited at NCBI CGAP would be enlightening. For example, is the KS gene expression pattern more comparable to HUVECs with or without VEGF stimulation (a major hypothesis in KS pathogenesis)? Since the KS SAGE results can be reanalyzed in silico, this is not a fatal flaw but it does dramatically limit the conclusions about pathogenesis that can made.

2. RT-PCR is not sufficient for this study. Either the SAGE abundance is inaccurate or the RT-PCR results are inaccurate. How was semi-quantitation done (e.g. by serial template dilution)? The limited difference on RT-PCR is characteristic for the nonquantitative nature of RT-PCR and is a likely source of this error, resulting in underestimation of the true differential gene expression. This should be resolved by northern blotting, or if starting RNA is limiting, by quantitative real-time
RT-PCR.
3. Five genes were studied to find a specific marker for KS based on their differential expression in skin and in publicly deposited CGAP SAGE libraries. A better comparison for this purpose would be PBMC libraries. Due to the large number of tags differentially expressed, it is likely that other genes not considered in this analysis might provide a more robust PBMC marker for KS.
4. The unique properties of SAGE, namely identification of previously unknown transcripts, is not taken advantage of. A brief description of transcripts 20, 21 and 30 in the supplement would be valuable, especially if differential expression is confirmed.
5. The raw SAGE counts should be deposited (either at NCBI or on a local server) to allow future comparisons.

**Competing interests:**

None declared.