Reviewer’s report

Title: Pathway aberrations of murine melanoma cells observed in Paired-End diTag transcriptomes

Version: 1 Date: 18 February 2007

Reviewer: Carl Anderson

Reviewer’s report:

General
This manuscript, “Pathway aberrations of murine melanoma cells observed in Paired-End diTag transcriptomes”, reports a transcriptome analysis of melanoma cells using the Paired–End diTaging method (GIS-PET) for 5’ and 3’ quantitative transcript analysis developed by the Singapore group. The GIS-PET method has several advantages compared to more standard techniques for transcriptome analysis such as SAGE or hybridization to microarrays. First, both the 5’ and 3’ ends of a transcript are simultaneously tagged and mapped back to the genome permitting location of both the transcript start and polyadenylation sites. Second, since the method is sequence-based, the data are highly portable and instrument independent, in contrast to most microarray data.

This manuscript reports changes (up and down) in the transcriptome of a murine melanoma cell line compared with three non-melanoma transcriptomes that impact numerous metabolic pathways. Some salient points uncover in this excellent study are that many of the differences in transcripts correspond to metabolic or cell cycle pathways in the KEGG database; however, developmental pathways also were altered that presently are uncharacterized in the KEGG database. Perhaps the most striking finding is that mitochondrial activity and putative mitochondrial permeability in melanoma cells through changes in the levels of transcription of pathway genes. The data they present support their hypothesis that melanoma cells exhibit downregulation of mitochondrial–mediated apoptosis and perhaps more dependence of early in situ melanoma metastasis on angiogenesis. Another important feature of the manuscript is the finding of alternative mRNA start and polyA addition sites as a common feature in melanoma cells. However, two cautions should be mentioned. First, only one melanoma cell line was analyzed and the generality of the findings thus need to be confirmed in other melanomas; second, it is not clear how many of the reported changes reflect adoption of the cells to in vitro culture conditions.

In summary, the authors have used a relatively new technique globally analyze changes in transcription associated with the development of melanoma. The authors report important findings that deserve to be disseminated to a wide audience. The manuscript is well written, clear and concise.

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Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)
None

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Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)
Attention should be paid to proper and consistent use of protein and gene names. For example the p53 protein is encoded by the TP53 [italics] gene in humans and the Trp53 [italics] gene in mouse.

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Discretionary Revisions (which the author can choose to ignore)

Page 3, line 4 – what is meant by “were more localized”?
Page 4, line 14 – “It extracts short, [positionally defined], tag signatures….” is clearer.
Page 6, first two lines are confusing as the PET counts do include duplicates and higher counts. Perhaps the word “samples” needs to be better defined.
Page 8 – Methods – state the cell lines are murine; define “LIF”.
Page 14, line 14 and elsewhere—references to the hypergeometric distribution method should be included. Is there a reference to how the s0 was empirically determined?
Page 23, end of paragraph – While it is possible that ROS levels remain high but activate survival pathways, this does not seem to be an alternative explanation for global changes in transcript levels of glutathione pathways.
Page 23, line 10 – “post-transcriptional regulation of P53 in melanoma cells” implying changes in mRNA
degradation? The description relates to the regulation of p53 at the level of transcription. Later – ARF: “degradation of p53” would be better than “repression” or “derepression” which usually refer to transcription.
Page 24 – changes in cell cycle gene transcripts. Are the growth rates and cell cycle compartments of the cell lines comparable?
Page 27, line 3 – reference for “previous reports”?
Page 29, last line – “increased” rather than “activated”.

What next?: Accept after minor essential revisions

Level of interest: An article of outstanding merit and interest in its field

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:
'I declare that I have no competing interests'