Comments for the Author

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Epigenetic and Transcriptional Architecture of Dendritic Cell Development
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Supplemental Files: 1

Reviewer 1 Comments for the Author
Reviewer 2 Comments for the Author

Reviewer 1 Comments for the Author...

Lin et al report analysis of genome-wide datasets for mouse dendritic cells, specifically gene expression, histone modification ChIP-Seq and Pu.1 ChIP-Seq data. The paper was submitted to BLOOD as an eBLOOD submission, and I have evaluated it from three perspectives (see below). In my view, the paper falls short on all three.

Perspective 1: Scientific Advances / New Biological Messages
There really is very little in this paper that could be classified as new scientific insights. It is well established that certain histone modifications correlate (positively or negatively) with gene expression, and Pu.1 binding maps have been reported before in dendritic cells and also MPPs. The network diagrams in the paper are speculative, since there is no experimental validation of any of the links in any of the networks.

Perspective 2: Bioinformatics/Data analysis
The bioinformatic methods appear sound, but not innovative.

Perspective 3: Value as a Resource
The authors are correct in pointing out that the paper last year from Ido Amit's

The authors are correct in pointing out that the paper last year from Ido Amit's lab did not include dendritic cells in their overall analysis. However, mouse dendritic cells have been the workhorse of genome-scale analysis for both the Amit and Regev labs, and there are very large datasets publicly available from their other papers. This current paper adds information on some of the progenitor populations, and this is of value to the community. However, this is really only a couple of new samples, and the question must be asked whether this would warrant a BLOOD paper. Of note, the expression data in the current submission are from a previous paper by the same group, which already included detailed microarray expression analysis. The current paper therefore in fact presents a reanalysis of old data.
Lastly, the value of a resource very much depends on its accessibility/usability by the wider community. No real effort is made in this paper (other than a GEO submission). Common practice for a resource paper would be the setting up of a dedicated web browser with visualization and search facilities.

Some further specific comments:
1) Title at top of page 10: There is no experimental data in the paper that would support such a strong statement on "instructive role"

2) Page 12 "Pu1 centered regulatory circuitry": If Pu.1 is the only factor for which the authors performed ChIP-Seq at the various stages, why should the reader be surprised that they end up with a Pu.1 centered network. It seems a self-fulfilling prophecy to me.

3) Page 12, same paragraph: The authors state Pu.1 activates, but show no experimental evidence for this. I expect they will have thousands of Pu.1 binding events, many in genes that do not change expression. The relationship between Pu.1 binding and gene expression changes is likely complex. Just because a gene changes expression and has a Pu.1 peak, this does not prove activation by Pu.1.

Reviewer 2 Comments for the Author...

In this manuscript, the authors aim on identifying regulatory principles driving dendritic cell (DC) development. To achieve this aim, they analyse histone marks associated with active and repressive chromatin, and PU.1 occupancy using genome-wide data sets of in vitro differentiated DCs and their progenitors. They use these data to assemble PU.1-centered regulatory circuitries providing a dynamic transcription factor hierarchy which they claim orchestrates the step-wise differentiation process of DCs.
This is a well written manuscript and an interesting topic. However, the main criticism of this reviewer is that the work contains way too many assumptions without any or very little functional testing. As such, the key statements are purely based on correlations and lack experimentally proven data. It would have been very easy and straight forward to functionally evaluate at least some of the predictions. For example, the authors should have performed siRNA-mediated

knockdown of PU.1 and some of the other herein identified DC transcription factors, to test if and how this affects their predicted networks and circuitries. Along the same line: to convincingly address if a DNA region functions as an active enhancer element, some kind of a reporter assay needs to be performed. It is not enough to simply look at a few associative chromatin marks. Also, to understand which gene is regulated by a particular enhancer, 3C or 4C experiments need to be performed. Moreover, increased PU.1 ChIP-seq peak numbers or intensities during the differentiation towards DCs does not prove or even suggest that PU.1 functions as a pioneering factor. Where are any functional experiments supporting such a key statement?
In addition to the lack of functional experiments, some of the computational analyses are not well controlled. For example, the authors restrict the analyses of cooperating transcription factor motifs to ChIP peaks close to genes with changed expression, and control this against randomly selected genomic 500 bp regions. These regions were not even matched for mappability or sequence complexity. More appropriate controls would be to take unchanged peaks close to differentially expressed genes or changed peaks close to unchanged genes.
Collectively, it is the conclusion of this reviewer that without such kind of functional evaluation, the data are too speculative and preliminary.