Reviewer's report

Title: Gene expression analysis in human osteoblasts exposed to dexamethasone identifies altered developmental pathways as drivers of osteoporosis

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Reviewer: Wilma T. W.T Steegenga

Reviewer's report:

General
The authors present a manuscript with a combined microarray and bioinformatics analysis of dexamethasone-induced gene expression in human osteoblasts. A focus on gene functions revealed that genes with known functions in development may be modulated by dexamethasone. Of 4 genes related to Wnt signaling, such modulation was confirmed by RT-PCR. The authors conclude from their experiments that alterations in these developmental genes drive osteoporosis.

Osteoporosis is a frequently occurring disease in our ageing society and research aiming to elucidate the molecular mechanism causing this disease is of high importance. In particular, glucocorticoid-induced osteoporosis is an important cause of osteoporosis and deserves attention.

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Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

1. The title of the manuscript is not appropriately chosen. Although putative dexamethasone responsive genes are found, the manuscript does not provide any functional data. Therefore, it can not be stated that the genes (or developmental pathways) are indeed drivers of osteoporosis.

In the abstract the authors mention that they have used "an integrated genomics and computational biology to identify key genes and gene clusters whose differential expression drives osteoporosis". However, what they provide in this paper is a list of genes and gene clusters that might be involved in the regulation of dexamethasone-induced osteoporosis and again functional data are lacking to support this conclusion. Furthermore, since the dexamethasone treatment was only applied to osteoblasts it can not be excluded that, at least part of the regulated genes, are involved in a general cellular response to dexamethasone treatment and do not represent an osteoblast-specific effect.

The conclusions drawn in this paper should much more carefully be formulated.

2. The exact experimental design is not clear. Do the cells receive fresh medium after o/n incubation on serum-free medium or is dexamethasone added to the cultures? If fresh medium is added to the cells appropriate controls are required in the microarray experiment (non-dexamethasone-treated samples of each time point).

3. A gene profiling study on dexamethasone-treated osteoblasts as a model for glucocorticoid-induced osteoporosis is not new. A similar study has been published by Leclerc et.al., 2004, J Mol Endocrinol, who studied dexamethasone-induced gene expression in the murine osteoblast cell line MC3T3. The authors should at least review their data with respect to the already published data.

4. The mRNA expression levels in figure 4 are shown relative to control. However, the authors do not show the gene expression levels on similar time points in the absence of dexamethasone. For this reason, it can not be concluded that the responses are specifically triggered by dexamethasone. To strengthen the hypothesis that dexamethasone-induced expression modulation of development related genes may be associated with osteoporosis, the authors should include data showing that dexamethasone treatment indeed affects osteoblast differentiation in their experimental setup.

5. On page 8, it is mentioned that 106 genes were development associated genes. In table 1, a number of 39 developmental genes are shown as being up-regulated. So apparently, 67 developmental genes are down-regulated. On page 7, however, the authors state that activation of developmental pathways may undermine osteoblast activity in osteoporosis. Since more developmental genes are down-regulated, it may thus also be reasonable to conclude that inactivation of developmental pathways is at the basis of dexamethasone-induced osteoporosis. It would be of interest to include a table of down-regulated genes as well. Furthermore, based on KEGG pathway analysis, a distinction could be made between pathways that
may be either activated, or inactivated by dexamethasone.

6. The results of figure 4 should be compared with the microarray data. A comparison between table 1 and figure 4 suggests that a discrepancy between the results obtained by the two assays is present. Furthermore, the authors should indicate in the text that frizzled 2 and 7 are not present in table 1 since these genes are identified as down-regulated genes (see also note 5).

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)
1. The graphs in Figure 1 are not labeled, abbreviations are not explained. Figure 1 and Figure 2 do not contribute to a biological understanding of the effect of dexamethasone on osteoblasts.

2. In the Results section, some known effects of the Wnt pathway on bone are mentioned, but referencing is very poor or completely lacking.

3. The authors mention in the abstract that they identified 500 osteoblast genes. In the Results section it is mentioned that 31, 83, 130, and 300 genes are modulated at the different time points measured. It is not clear whether these are distinct genes, or if there is any overlap.

4. The manuscript contains very many typographic errors. The usage of capital or lower case letters for gene names is not consistent and many incomplete or incorrect sentences are present in the manuscript.

Discretionary Revisions (which the author can choose to ignore)

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: No

Declaration of competing interests:

I declare that I have no competing interests