Reviewer's report

Title: Silencing Dkk1 Expression Rescues Dexamethasone-Induced Suppression of Primary Human Osteoblast Function

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Reviewer: Ya-Wei Qiang

Reviewer's report:

In this manuscript, the author reported that glucocorticoid agent dexamethasone (Dex) attenuates human osteoblast differentiation, and concomitantly, inhibition of canonical Wnt signaling pathway in these cells. The authors also demonstrated that that interfering with Dkk1 expression alleviates Dex-disturbed human osteoblast function. The authors thus concluded that Wnt signaling plays a key role in regulating glucocorticoid- triggered osteoporosis. While most data in current study are not particularly novel, since glucocorticoid-induced damaged Wnt signaling as the one of mechanism underlying the Dex-induced osteoporosis has been well established in vitro human osteoblast and in vivo mouse model (Ohnaka et al, Biochemical and Biophysical Research Communications, 2004 and 2005, Wang et al, Endocrinology, 2008), more evidence should be provided to validate the biological role of Dkk1 in glucocorticoid-induced impaired osteoblast function in human primary osteoblasts by addressing the follow questions and concerns:

Major Compulsory revision

1. There are concerns about the experimental design for determining the effect of Dex on Osteoblast differentiation and the role of silencing Dkk1 expression in this process. The time for inducing osteoblast differentiation and then measured by ALP and Alizarin Red-S (ARS) analysis to determine the role of knockdown Dkk1 expression in Dex-impaired to osteogenetic differentiation in this study is much shorter compared with that of well-established ALP and ARS, which has been utilized by many studies to determine osteoblast differentiation in both mouse and human in vitro cells model (Jaiswal et al, Journal of Cellular Biochemistry, 1997: 64:295; Tondveau et al, Cytotherapy, 2004, 6:372, Wang et al Endocrinology 2008, 149:1793, Qiang et al, Blood 2009, 113, 43019). Based on these studies and our experience to determine the osteogenesis differentiation, it takes 12 to 21 days for the positive in ARS and at least 72 hours for ALP activity be detected when human mesenchymal stem cells are cultured in medium containing osteoblastic differentiation media. In this study, the authors measured the ALP activity and calcium deposit using ARS analysis starting from 4 hours and the long time was at 48 hours after treatment of the cell with Dex. It is impossible that osteoblasts are able to differentiation in so short periods of time in vitro culture system due to regulating Wnt signaling or Dkk1. Furthermore, it is not clear if any osteogenetic inducing reagents were utilized or not. Moreover, since Dex can directly induce osteoblasts to undergo apoptosis (Wnag et al,
Endocrinology 2008, 149:1793), it is necessary to clarify the cause of Dex-decreased activity in ALP is due to its induced apoptosis or inhibit osteoblast differentiation by targeting Wnt signaling and Dkk1.

2. Since the author has demonstrated Dex inhibited beta-catenin and TCF transcriptional activity, it is necessary to detect effect of Dex on Dkk1 expression in these cells in order to clarify if Dex direct has effect on b-catenin degradation or via target Dkk1.

3. It is not clear if silencing Dkk1 expression had functional suppression in Wnt/beta-catenin function in the studies. The beta-catenin measured by immunofluorescent staining analysis for beta-catenin and TCF transcriptional activity by luciferase report assays should be done after silencing Dkk1.

4. To validate the Dkk1 biological function in Dex-induced impairment of osteoblast function, neutralized Dkk1 antibody should be used to determine the effect of blocking endogenous Dkk1 function in ALP and calcium deposit in human osteoblast. The experiments that put an emphasis on biological role of Dkk1 in glucocorticoid-impaired osteoblastic function could add the credits for this study since it is a open question in human osteoblast although it has been reported in mouse model, and considering that the deleterious effect of Dex on Wnt-signaling is not novel in human osteoblast since it has been reported by a previous study (Ohnaka et al, Biochemical and Biophysical Research Communications, 2005).

5. In discussion, the author should summarize the novel findings in the study.

Discretionary revision

1. The protein level of Dkk1 should be measured after silencing Dkk1 expression in human osteoblast cells. This is now easy, since there is a commercial available kit (R & D system).

2. To further support the conclusion, recombinant Wnt3a or Wnt3a protein (increased Wnt signaling) should be used to see if increasing Wnt signaling could rescue Dex-impaired osteoblast function.

Minor Essential Revision

1. In Figure 3a and Figure3b, based line TCF transcriptional activity measured by luciferase activity in the report constructed transfec human osteoblast cell are obviously different. Over 3000 RLU are featured in Figure 3a, at zero hours (control), but less than 1500 RLU in control (with L-cell CM). What causes this discrepancy?

2. In my printed MS, all figures missed labeled numbers (such as figure 1, 2.). The author should label them in order.

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

Quality of written English: Needs some language corrections before being published
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.