Reviewer’s report

Title: The Effects of Low-dose X-irradiation on Osteoblast-like Cells in Vitro

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Reviewer: John Hawse

Reviewer’s report:

This manuscript describes the effects of low dose irradiation on osteoblast cells in vitro. The authors have found that low dose irradiation decreases proliferation rates in a dose dependent manner 5-6 days following exposure but does not alter the cell cycle profile or result in changes in apoptosis. However, low dose irradiation was shown to increase alkaline phosphatase positive cells and increase mineralization rates of MC3T3 cells. This was accompanied by significant increases in the expression of osteocalcin, Cbfa1 and OPG with a concomitant decrease in RankL expression levels. While this manuscript expands upon previous studies, provides insight into the effects of low dose irradiation on osteoblasts and addresses a clinically relevant subject, a number of revisions are necessary prior to publication.

Major Compulsory Revisions:

1). The authors state both in the introduction and in the discussion that previous reports have demonstrated that moderate to high doses of irradiation negatively affect osteoblast proliferation and differentiation and acknowledge that their present results are in contrasts to these published reports. The authors also state that identifying the range of irradiation which begins to negatively impact osteoblast cell populations is necessary. However, they do not provide any data to show that increasing doses of irradiation do negatively affect osteoblasts. For these reasons, the authors should add a few more increased doses of irradiation to the experiments provided in this manuscript to address these important issues and to confirm that low dose irradiation is actually beneficial.

2). It is not apparent, nor explained, why decreased proliferation was observed at day 5 and 6 following irradiation. Since no changes in the cell cycle or apoptosis levels were observed, these data do not make sense. Typically, if cells are not dying, it would be expected that the curve would eventually plateau, but not decrease. This issue should be explained.

3). With regard to the above comment, if the decrease in proliferation is real, a possible explanation would be increased cell death. However, the authors did not examine the levels of apoptosis at days 5 and 6 when these decreases were observed. Instead, they only looked at days 1-3. The authors should repeat the BrdU and apoptosis assays on days 4-6 to match their proliferation data.

Minor Essential Revisions:
1). The composition of the cell lysis buffer used for protein extraction should be described in the methods section.

2). Why were the protein levels of OPG and RankL not analyzed as was done for OCN and Cbfa1? This data would make a nice addition to confirm the gene expression results.

3). There are numerous grammatical errors throughout the manuscript that need to be addressed prior to publication.

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

**Quality of written English:** Not suitable for publication unless extensively edited

**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.