**Reviewer's report**

**Title:** Regulation of MCP-1 secretion in a novel bone-tumor coculture model

**Version:** 1  **Date:** 28 August 2008

**Reviewer:** Ling Qin

**Reviewer's report:**

**Major Compulsory Revisions**

This manuscript by Shiller et al aims to establish a novel coculture system to study the interaction between bone microenvironment and tumor cells. If succeed, this system has important applications in cancer field because current in vitro coculture models can only study interaction between two types of cells either in the same well or separated by permeable membranes and thus they lack three-dimensional architecture and multiple cell interactions.

In this new coculture system, an intact neonatal femur was immobilized at one side of a well in a 6-well plate and cancer cells were plated on the opposite side of the well containing cell culture medium. Immediate concerns about this system are whether cells inside bone marrow cavity can get enough nutrients from culture medium outside of bone and whether those cells can interact with tumor cells outside of bone. To address the first concern, authors used live/dead viability kit and found cells in the outer bone layers were still alive after 2 days in culture, possibly due to their direct contact with culture medium. However, the cells inside bone marrow cavity showed a dramatically increase in apoptosis number after 2 days of culture, indicating that those cells were dying during coculture period. In Figure 2F, only apoptotic cell number per field was presented. It is important to show the percentage of apoptotic cells, a better indicator of severity of cell death. This can be done by using the same method for Figure 2D and 2E and then counting stained cells in both pictures. If the majority of bone marrow cells are apoptotic after 2 days, then 48 hrs is not a good time point for coculture and authors should find out the best time point when most bone marrow cells are still alive and use that time point for subsequent experiments. If it is true that most bone marrow cells died after 2 days, an alternative explanation for the observed synergistic regulation of MCP1 in the coculture system is that it is due to the interaction between tumor cells and cells on the outer bone layer.

To address the second concern whether bone marrow cells can interact with tumor cells outside of bone, authors used bone marrow depleted femurs in the coculture system and found the synergistic effect on MCP1 level was abolished. One question regarding this experiment is why authors used 24 hr time point instead of 48 hr. In the previous experiment using intact femur, authors only observed strong synergistic effect on MCP1 level at 48 hr but not at 24 hr. Authors did not explain in detail how to flush out bone marrow. We normally cut both ends of femur and then flush out bone marrow cells. So compared to intact
femur, bone marrow depleted femur not only lacks bone marrow but also epiphyses. It is possible this effect is due to the lack of epiphyses which contain a lot of bone cells as shown in Figure 2A and B. This fits well with the aforementioned alternative explanation. Taken together, these two major concerns need to be addressed before accepting this manuscript.

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

**Quality of written English:** Acceptable

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**

I declare that I have no competing interests.