Reviewer's report

Title: Cooperative effects in differentiation and proliferation between PDGF-BB and matrix derived synthetic peptides in human osteoblasts

Version: 2 Date: 3 September 2011

Reviewer: Rainer Meffert

Reviewer's report:

General:

The authors report on the effects of combining platelet-derived growth factor-BB (PDGF-BB) and synthetic ECM-associated proteins in 2-D culture of osteoblast-like cells. The study is well designed and most conclusions drawn appear to be solidly based on the results. The comments below should be addressed before publication is considered.

Minor Essential Revisions

1) Abstract, p.1: The abstract should be shortened, especially the Materials and Methods section is far too detailed.

2) M&M, p.5: Stimulation of osteoblast-like cells: PDGF and the peptides are the key components of the study, however, information on these factors is missing in the M&M section. It should be detailed where the substances were obtained from and how they were dissolved and applied to the culture. Furthermore, each substance was applied only in one concentration. It should be clarified on which information the choice of these concentrations was based.

3) M&M and Figs. 1-3: Please clarify the data shown in Figs. 1-3. Currently, the y-axis is named "cells/area". However, in the Materials & Methods section (p.5-6), it is stated that staining with Ki67 antibody for proliferating cells and staining with methyl green for total cells was performed and that cells were counted after both stainings. What exactly are the cell numbers in the figures and in which area the cells were counted?

4) Results, p.7: The paragraph regarding calcification, i.e., the key results of the study, should be modified. Currently, it is only stated that all three combinations of PDGF with the different peptides achieved an increase of calcification as compared to the control group receiving no stimulating factors. However, judging from Figs. 4-6, it appears as if there were clear differences between the peptides, which also should be described. For the combinations of PDGF with the peptides p15 (Fig. 4) and TP 508 (Fig. 5), no significant differences were achieved as compared to PDGF alone. In contrast, for the combination of AC 100 with PDGF, a clear synergistic increasing effect is apparent as compared to either factor alone (Fig. 6). This important finding should be highlighted in the Results. Furthermore, possible reasons for this difference between the combination
groups should be discussed in the Discussion section.

5) Discussion, p.9, l.6: It is stated that, “The calcification was accompanied by an increase in the gene expression of the matrix components collagen I and osteocalcin.” However, in the Results section (p.8, l.7), contrasting results were described: “..., a reduced collagen I expression was found, especially when combinations of PDGF with AC 100 or p15 were used.” Please clarify.

Technical

1) Please double-check all citations in the reference list and the text. Currently, many references appear twice in the list, e.g., the same papers are #20 and 22, #21 and 23, #32 and 33, #41 and 42, #47 and 50, #48 and 49, #52 and 55, #53 and 54.

2) The name of the medium should be corrected to “... HAM’S F12 ...”, instead of “... HARMS F12 ...”. (p.1, l.16; p.5, l.7).

3) Other typos or expressions to be changed include:
   - p.1, l.12: It should be, “... could increase proliferation ...”, instead of, “... could increased proliferation ...”.
   - p.2, l.5: “alkaline phosphatase (ALKP)”, instead of, “Alcine Phosphatase (AP)”.
   - p.6, l.2: “Counter stain ...”, instead of, “Counter stein ...”.
   - p.7, l.2: “Mann-Whitney U test”, instead of, “Man Withney U test”. Same spelling error occurs also in the abstract, but there the Statistics should be deleted anyway (see comment 1).
   - p.8, l.8: “AC 100 or p15”, instead of, “AC 100 of p15”.
   - p.10, l.2: The first sentence in the Conclusions section is unclear and should be deleted or clarified.
   - p.10, l.5: “PDGF-BB”, instead of, “PDGF-ββ”.
   - p.10, l.6: change to, “healing of osteoarthritis defects”.
   - p.10, l.10: change to, “are needed to be performed”.
   - p.11, l.1: “Competing interest”, instead of, “Compending interest”.

4) In general, parts of the text would benefit from some language corrections before being published.

**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'