Reviewer's report

Title: Modelling NF1 tibial dysplasia and its treatment with lovastatin.

Version: 1 Date: 28 April 2008

Reviewer: Juha Peltonen

Reviewer's report:

The authors utilize Nf1Prx1 mice which are predicted to harbor NF1 deficient mesenchymal stem cells. Healing of experimental tibial trauma was followed in control and in gene modified mice, with and without lovastatin treatment. The healing process was analyzed in three time points using histochemistry, 3D micro CT, in-situ hybridization for type I collagen, Runx2 and osteopontin, and western transfer analysis for p-p44-42 and p44. The mutated animals, experimental fracture model, and the analyses have been described earlier. The results as such are based on credible documents with few exceptions.

The main result is that lovastatin seems to improve bone healing in Nf1Prx1 mice. The main conclusion outlined on page 3 that “Presented experimental model constitutes a valuable tool for the pre-clinical stage testing of candidate drugs, targeting Nf1 associated bone dysplasia” is founded. The Title of the MS is well conceived avoiding speculation.

This reviewer has the following criticism and suggestions for improvement:

- Some conclusions and introductory elements apart from those mentioned above are too speculative; these are exemplified below, and certainly recognized by the authors.

- The methods and introduction need more details, even though properly referenced. A study has to be independently readable. Just for example, even the Nf1Prx1 mice need to be introduced in more detail in the text, and the sequence specificities of the probes for in situ hybridization lack necessary details. Various expressions are not accurate, for instance: “RNA probes” on page 6 apparently refer to cRNA probes.

Abstract background (page2): Quote “Here we report results of experiments in which we used a cortical bone injury model to simulate Neurofibromatosis type I (NF1) associated bone changes, in particular pseudarthrosis”. This model system may have no direct relevance to NF1-associated osseous lesions, and the statement goes beyond clinical rationale. The manuscript does not tell, or at least it is not easily detectable whether the mesenchymal progenitors in Nf1Prx1 mice were heterozygous or homozygous for Nf1 inactivation. Even if it is evident from the previous publications, this information must be given here.

“During normal bone repair, fibroblasts from the surrounding connective tissue along with mesenchymal progenitor cells from the periosteum”… This statement is not solid; the origin of cells becoming osteoblasts in fracture healing has not
been proven in detail.

The pharmacological background of lovastatin should be explained in more detail on pages 4-5. Even though quite correctly stated “Statins are inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A reductase, broadly used for reduction of serum cholesterol. As statins inhibit the initial enzyme of mevalonate pathway, they also reduce prenylation and farnesylation of signalling molecules, such as Ras and Ras-related proteins”, important information is missing on drugs in clinical for osteoporosis and interfering the same mevalonate pathway, but on a different point. These drugs, bisphosphonates, mainly interfere with osteoclast function, which is also relevant to bone dynamics. The Discussion notes (data not shown) that the number of osteoclasts at the site of experimental injury in mutant animals was not increased. It is unclear how the osteoclast count was performed. Also the mere number of osteoclasts as such does not necessarily correlate with osteoclast function, a factor which may potentially interfere with the results. It should also be highlighted that osteoclasts in this experimental model may not be Nf1 deficient.

**Which journal?:** Appropriate or potentially appropriate for BMC Medicine: an article of outstanding merit and interest in its field

**What next?:** Accept for publication in BMC Medicine after minor essential revisions

**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’