Reviewer’s report

Title: Nestin expression in osteosarcomas and derivation of nestin/CD133 positive osteosarcoma cell lines.

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Reviewer: Andy Peng Xiang

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The study by Veselska et al. investigated the nestin expression on the osteosarcomas samples by immunohistochemistry and found most of the samples showed various nestin expression patterns ranging from very sporadic occurrence to an overwhelming proportion of cells with strong positive staining. The authors also derived osteosarcoma cell lines and showed the Nestin and CD133 expression on three of them.

In general, such information is novel and may be useful in future clinical practice. There are, however, some important limitations in this study and need clarification/further interpretation.

1. The significance of this study is hampered by the small sample sizes (number of cases) and it is in doubt whether the conclusion can be generalized.

2. The authors have not described the biological significance of the nestin expression on the osteosarcoma, for example: the correlation between nestin expression and malignancy or clinical outcome; the data presented are too preliminary to answer these questions;

3. In this study, only immunohistochemistry/immunocytochemistry was applied to investigate the expression of Nestin. The authors do not provide any experimental evidence documenting that their antibody used in the immunohistochemical studies recognizes nestin (Western Blots); it would be helpful to study nestin expression in fresh tumors using western blot.

4. For the osteosarcoma cell lines, the authors provide few specific characteristics about the cell line, for example, how long have you expanded this four osteosarcoma cell lines in vitro? how about the karyotype and the doubling time of these cell lines? How about the tumorgenicity of these cells in nude mice?

5. The authors should provide the percentage of nestin positive or CD133 positive cells in the cell lines?

6. what is the biological significance Nes+/CD133+ cells in the cell lines ? cancer stem cells? The authors need to provide more information on this issue.

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

Quality of written English: Acceptable
**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**

I declare that I have no competing interests