Reviewer's report

Title: The genetic basis for inactivation of Wnt pathway in human osteosarcoma

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Reviewer: Prakash Babu Phanithi

Reviewer's report:

The current study attempted to find out frequently altered genes in the tissues samples of human Osteocarcinoma. The authors used techniques such as microarray-based comparative genomic hybridization (aCGH) and transcriptome sequencing (RNA-seq) followed by pathway analysis. The major finding of this particular study is deletions observed in the Wnt signaling pathway genes i.e. significant gene copy number deletions, low mRNA expression, negative nucleus #-catenin expression, and low level expression of cyclin D1/c-myc. Based on the above observations authors stated that Wnt signaling pathway is inactive after Osteocarcinoma. Detailed studies on the role of Wnt signaling pathway might help to understand the mechanism of tumorigenesis in Osteosarcoma. However, authors failed to address many important questions as mentioned below in order to demonstrate Wnt signaling inactivation in Osteosarcoma.

Comments to authors:

1. First, there are many sentences/statements are repetitively mentioned though out the manuscript and also poor grammar as well. Authors strongly suggested to proof read the manuscript thoroughly.

2. What is the procedure followed to collect osteosarcoma tissue samples for processing? Please explain in detail that how frozen samples been processed for RNA studies.

3. What is the section thickness for immunostaining? Please explain the procedure briefly, starting from the sectioning to microscopy. What is the scale for microscopy images?

4. Did the authors counterstain the sections showed in figure. 4? What is the cellular localization for proteins Wnt1, #-catenin, c-myc, and cyclin D1?

5. What is the status of Wnt1, #-catenin, c-myc, and cyclin D1 staining for control sections?

6. The data showed in figure. 4 should be supported by performing western blot for at least two important proteins of Wnt signaling pathway.

Overall impression: Major revision

Level of interest: An article of limited interest
**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 interest