Reviewer’s report

Title: The correlation of WWOX, RUNX2, and VEGFA protein expression in human osteosarcoma

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Reviewer: Andrew E Horvai

Reviewer’s report:

In this manuscript, the authors investigate the gene copy number changes (by aCGH and FISH) and protein expression (by immunohistochemistry) of three genes (WWOX, RUNX2 and VEGFA) possibly involved in osteosarcoma tumorigenesis. The genomic results and immunohistochemical expression do not correlate for each gene, but the results are nonetheless interesting and worthy of publication.

The hypothesis is clearly defined and the methods used are appropriate to answer the questions posed.

Minor Essential Revisions

1. Although the manuscript is understandable, it might benefit from careful review of grammar by a native English speaker. It contains numerous minor grammatical errors (subject-verb agreement, preposition omitted, spelling etc.).

2. I notice from Table 1 that 4 osteosarcomas were stage I, which by AJCC/UICC criteria implies they were low grade. Was there any difference in the aCGH pattern of these low-grade osteosarcomas? Since previous studies have suggested 12q13-15 amplification in low grade osteosarcomas, it might be worth mentioning in the discussion how the authors’ data on the low-grade tumors relates to those prior findings and to reference the literature on 12q13-15 in osteosarcoma.

3. I’m confused by Figure 1 A. The legends reads ” line-plots denote the estimated copy number value of 20 specimen” Is Figure 1A an overlay of ALL cases tested by aCGH? Or an average of gains and losses among all the cases? Or is this just a representative genome from one case? Any of the above would be fine, it’s just not clear from the figure legend which one it is.

Discretionary Revisions

1. Figure 2 is not very convincing of amplification. I do not doubt the authors’ results, but many cells seem to have equal numbers of orange and green, some have orange but no green (and thus probably should be excluded from analysis). It might be better to just demonstrate one nucleus with clearly increased numbers of orange signals relative to green signals.

2. In the results, the authors state “Interestingly and surprising, in our 10 osteosarcoma samples performed aCGH detection, all 6 osteosarcoma samples with RUNX2 amplification were observed negative protein expression.” In the
discussion, this is addressed with the following statement “So our data which demonstrate lack of significant positive relation between RUNX2 gene amplification and frequent protein expression might suggest that in osteosarcoma the increased expression of RUNX2 is likely to be driven by other factors in addition to gene amplification.” This discussion point would apply if there was no amplification and the cells overexpressed RUNX2 by immunohistochemistry. But, it does not touch on the disconnect between genetic amplification and undetectable expression by immunohistochemistry. Since this is such an unexpected finding, it would be useful to the readers if the authors proposed some explanation(s).

**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:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**

I declare that I have no competing interests'