Author’s response to reviews

Title: CDK1 and CCNB1 as potential diagnostic markers of rhabdomyosarcoma: Validation following bioinformatics analysis

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Author’s response to reviews:

Xianquan Zhan, Ph.D
Senior Editor
BMC Medical Genomics

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Dear Dr Zhan,

Re: Manuscript No. MGNM-D-19-00227, Submission of the revised manuscript entitled “The potential diagnostic markers of CDK1 and CCNB1 in rhabdomyosarcoma: validation following the bioinformatics analysis”

Thank you very much for your letter, questions and the editorial and reviewers' comments which we found very helpful in improving our manuscript. We have revised the paper according to the comments in a point-by-point format as shown below. The revised sections and sentences (not individual spellings and grammatical corrections) are in blue for easy identification.
In terms of English language editing, we have employed an English language service company to edit the whole manuscript.

In addition, all authors declare no conflict of interest to report.

We hope the revised manuscript is satisfactory and suitable for publication in your journal. Please let us know if further revisions are needed.

Sincerely yours,

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Responses to the reviewers’ comments:

Marcelo Alarcon, PhD (Reviewer 1):

In general, they deal with a topic of great relevance, there are some important aspects such as the introduction does not have much relation to the work presented, it should be reformulated to guide, for example, to talk more about CDK1 and CCNB1 as they relate to the disease
Response: Thank you for this comment. We have reorganized the background to make it closely integrated with the main content of this study and added CDK1 and CCNB1 as they relate to the disease in the revised manuscript.

Other aspects: All figures are very pixelated, which is why you can't get good conclusions.
Response: We are very sorry for these. Our original figures are large and clear, but their sharpness is reduced when they are converted to PDF format. We have tried our best to modify and upload new data.

Xiang Chen (Reviewer 2):

The authors performed differential expression and network analysis of gene microarray expression data in 66 samples of rhabdomyosarcoma based on existing bioinformatics tools. Two hub genes CDK1 and CCNB1 were selected as experimental subjects and inferred as potential
diagnostic and prognostic biomarkers. This is a routine analysis procedure, and its conclusions have been reported in previous studies. The main comments are as follows:

1. The author needs to review the existing rhabdomyosarcoma related biomarkers in the introduction part to further illustrate the originality of this paper's conclusion.
Response: Thank you for this comment. We reviewed existing rhabdomyosarcoma-associated biomarkers and added them in the background. In the revised manuscript you will see "Approximately 80% of all ARMS contains a fusion of the PAX3 or PAX7-FOXO1 gene, and this fusion-positive gene has been identified as a prognostic marker for RMS [2-6]. Other prognostic (e.g. MYOD1 mutation, RAS pathway mutation, NCOA2 or VGLL2 gene fusion) and diagnostic (e.g. intermediate silk proteins [desmin, vimentin and nestin]), muscle proteins [myoglobin and actin] and myocyte determinant-coding genes [MYOD1 and myogenin]) markers have also been found in RMS [3, 7]. Although these biomarkers play a considerable role in the diagnosis and prognosis of the disease, the 5-year survival rate of RMS patients is still lower than or equal to 20% [4-6]. Therefore, more efforts are needed to elucidate the molecular mechanisms underlying RMS development and determine the diagnosis and prognostic biomarkers of the disease.".

2. According to the paper, the statistical analysis on page 7 is derived from other tools, such as Limma, KM plotter, and is not performed separately and cannot be described as a separate part.
Response: We have eliminated the description of this section in the revised manuscript.

3. It should be explained how the final 2 hub genes were selected as rhabdomyosarcoma biomarkers.
Response: We have modified this section in the revised manuscript. In the revised manuscript you will see "The cell cycle-related molecules CDK1 and CCNB1 are the hub genes with the highest degree of connectivity, and these genes have been reported to be potential diagnostic or prognostic markers in a variety of tumours, such as glioblastoma malignancies [9], hepatocellular carcinoma [10, 11] and non-muscle invasive bladder cancer [12]. However, detection of CDK1 and CCNB1 in clinical samples of patients with RMS has not been reported. ".

4. Line 17 on page 5 regroupe-&gt;regroup
Response: Thank you for this comment. This sentence has been removed in the revised manuscript because the expression is not accurate.

Obul Bandapalli (Reviewer 3):

Li et al in their manuscript titled "The potential diagnostic markers of CDK1 and CCNB1 in rhabdomyosarcoma: validation following the bioinformatics analysis" have analyzed the DEGs between 82 samples of RMS tissues and 16 samples of normal striated muscle tissues and reported CDK1 and CCNB1 as potential diagnostic markers.
It is a good piece of work with validation experiment (IHC) on independent samples though low in number. Please perform at least q-PCR for the remaining 8 out of 10 hub genes and report the outcome so that it can give further strength to the study.

And also state that this study requires further validation in large cohorts as this is very small cohort before concluding with high confidence.

Response: We are very grateful for your suggestion. In the revised manuscript, we verified all hub genes by RT-PCR and added in the conclusions section. In the revised manuscript you will see "However, our results require further validation using larger cohorts."

The methods and results of RT-PCR are as follows:
Method:
Detection of the mRNA expression of the hub genes by RT-PCR
Total RNA was extracted from formalin-fixed paraffin-embedded RMS and normal striated muscle tissues using RNeasy FFPE kit (Cat No.73504, QIAGEN, Germany) according to the manufacturer’s protocol. The total RNA samples were reverse transcribed into cDNA by using SuperQuick RT MasterMix (for Real-Time PCR) (Cat no. CW2391M, Qiagen, China). RT-PCR analysis was performed with UltraSYBR Mixture (Low ROX) (Cat no. CW2601M, Qiagen, China) on the 7500 Real-Time PCR System (Applied Biosystems, USA). The primers were synthesised by Sangon Biotech and are presented in Additional file 1: Table S1. Experiments were repeated in triplicate. The relative expression levels of hub genes were normalised to those of GAPDH through the 2-ΔΔCt method.

Result:
RT-PCR validation of the mRNA expression of hub genes
The mRNA expression levels of the 10 hub genes were detected by RT-PCR to verify the results of the previous bioinformatics analysis. The 10 hub genes contain nine up-regulated genes (CDK1, CCNB1, CDC20, CCNB2, AURKB, MAD2L1, CENPE, KIF2C, and PCNA) and one down-regulated gene (HIST2H2BE). The RT-PCR data showed that although the trend of expression patterns of these 10 hub genes were consistent with the sequencing results, among these up-regulated hub genes, only CDK1, CCNB1, CDC20 and CENPE were found to be the most significantly up-regulated in RMS patients. In addition, mRNA expression of HIST2H2BE was significantly decreased in RMS patients (Fig. 6).