Author's response to reviews

Title: NMD inhibition fails to identify tumour suppressor genes in microsatellite stable gastric cancer cell lines

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Author's response to reviews: see over
Dear Editor,

Herewith I would like to submit our revised manuscript titled “NMD inhibition fails to identify tumor suppressor genes in microsatellite stable gastric cancer cell lines” by Buffart et al. for publication in BMC Medical Genomics.

We would like to thank the reviewer again for the comments and giving us the opportunity to resubmit the manuscript. A detailed reply to the reviewer’s suggestions is added. We hope that you find our revised manuscript suitable publication in your journal.

Thanking you in advance for your consideration, also on behalf of all authors,

Sincerely yours,

Beatriz Carvalho, PhD
Reply to the comments of reviewer 2:

For two of our candidate genes we were unable to completely sequence the cDNA of the whole gene due to PCR failures. We agree with the reviewer that we cannot exclude these two genes as false positive candidate genes if part of the sequence is missing. Since we didn’t manage ourselves to sequence those parts of the gene, we send out the genomic DNA to the company BaseClear (Leiden, The Netherlands) for sequence analysis. After designing new primers, the genomic DNA of these two genes was sequenced. Sequencing the genomic DNA of \textit{SLITRK6} was successfully performed. No mutations were detected, confirming that this gene also was a false positive hit. The gene \textit{INHBB} was again unsuccessfully sequenced. Although no PTC was detected in this gene we cannot fully confirm that this gene is a false positive hit. However, the message of the paper is unchanged. siRNA mediated inhibition of the NMD machinery yielded many false positive results in our hands. This is added to the manuscript, in the results and discussion section.

The other issue raised by the reviewer concerns the “natural substrates of the NMD”. This includes i.e. genes with alternative splice variants. In our study multiple genes containing alternative splice variants showed up as candidate genes harboring a premature termination codon. Due to the fact that they are natural substrates of the NMD machinery, we excluded them for further analysis for mutation since they would most probably be false positive hits. This is clarified in the manuscript.

Furthermore, to our knowledge, this is the first paper describing the GINI technology in gastric cancer cell lines. This makes it difficult to select genes from previous studies to check the behavior in our gastric cancer cell lines. Also, the gastric cancer cell lines used in this study are not commercially available and little is known about mutations in these cell lines. Therefore we do not have the knowledge beforehand which genes could be used as controls.