Author's response to reviews

Title: Somatic mutation analysis of MYH11 in breast and prostate cancer

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Author's response to reviews: see over
To the Editor,

Enclosed please find the revised version of the manuscript “Somatic mutation analysis of MYH11 in breast and prostate cancer” (MS:1482949605196577) submitted for possible publication in BMC Cancer. The manuscript has now been revised along the lines suggested by the reviewer. We appreciate the favorable comments and thank you for the possibility to resubmit our work. You can find below the comments and how each one of them is addressed.

Reviewer's report:

On the question of normal tissue contamination, even though it is clear that one can detect mutations in samples with 50% or more contamination, there are some caveats. It is not clear how the sensitivity of detection is affected by the level of the mutation. Particularly given the manual aspects of the checking process, the sensitivity can change from lab to lab and operator to another. I wish it would be possible to quantitate better the statement “from our experience one can detect mutations as low as 30-50%”. So how well is that done using the same process and manual checking procedure as done in this manuscript (from example using spiking experiments). I think this is particularly relevant since the paper rests on the negative result that no mutations were found. At a minimum, I think the authors should add a bit more details on the % cellularity of tumors to provide a loose measure of the confidence in the sequencing results. For example they can point to the percentage of tumors with (for example) >80% and 50% cellularity to indicate the percentage of tumors with high and medium level confidence.

Breast and prostate carcinomas are typically more dense than the surrounding stromal tissue or normal epithelium, so the cellularity in the malignant compartment is higher than in surrounding tissues. Therefore a tumor percentage 50% represents a tumor cell content > 50%. Out of 155 breast cancer samples 34 (21.9%) had a tumor content < 50% (approximately 40% of tumor cells). The prostate cancers contained typically >60% of malignant tissue. This information now appears in the manuscript (Materials and Methods, page 4:)
“Tumor samples were evaluated by a pathologist prior to DNA extraction and contained at minimum 30% of tumor cells. A total of 34 samples (21.9%) had a tumor content <50% (approximately 40% of tumor cells).”

In our experience even as low as 10% tumor cellularity is often sufficient to detect somatic mutations using AB 3730 BD3.1 sequencing chemistry and 5.1 sequencing analysis software. However, certain mutation types may be more challenging to detect using sequencing (and manual scoring) and may lead to false negative results. This is now discussed (Discussion, page 9).

“The negative results are unlikely to be due to normal tissue contamination, as the tumor cell content was typically >50%. Certain somatic mutations, undetectable by sequencing, may have been missed in this study.”

Please let me know if you need any additional information, or if there is any other assistance I can provide you with. I look forward to hearing from you soon.

Yours sincerely,

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